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# THE AMERICAN REVIEW OF TUBERCULOSIS

## *A Journal of Pulmonary Diseases*

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

## CONTENTS

The Growth of Intracellular Tubercle Bacilli in Relation to Their Virulence. G. B. MACKANESS, N. SMITH, AND A. Q. WELLS ..... 479
The Growth of Tubercle Bacilli in Monocytes from Normal and Vaccinated Rabbits. G. B. MACKANESS ..... 495
Correlation of Biologic Properties of Strains of *Mycobacterium* with Their Infrared Spectrums. III. Differentiation of Bovine and Human Varieties of *M. Tuberculosis* by Means of Their Infrared Spectrums. D. W. SMITH, W. K. HARRELL, AND H. M. RANDALL ..... 505
The Effect of Cortisone on BCG Infection in the Guinea Pig. KONRAD BIRKHAUG ..... 511
Viomycin Therapy in Human Tuberculosis. HENRY G. SCHAFFELD, BETTINA GARTHWAITE, AND J. BURNS AMBERSON ..... 520
The Use of Viomycin in Patients with Pulmonary Tuberculosis. JOHN D. ADCOCK, WINTHROP N. DAVEY, RAPHAEL R. HALEY, ROBERTS M. REES, AND WALTER A. MEIER ..... 543
Indications for Segmental Resection in Pulmonary Tuberculosis. LYMAN A. BREWER, III, HARLOW W. HARRISON, RODNEY P. SMITH, AND ANGEL F. BAI ..... 554
Descriptive Classification of Pulmonary Shadows. A Revelation of Unreliability in the Roentgenographic Diagnosis of Tuberculosis. R. R. NEWELL, W. EDWARD CHAMBERLAIN, AND LEO RIGLER ..... 566
Comparison Between the International Standard Tuberculins, PPD-S and Old Tuberculin. FLORENCE B. SEIBERT AND EMMA H. DUFOUR ..... 585
Comparison of Plasma Viscosity and Erythrocyte Sedimentation Rates in Pulmonary Tuberculosis. LOUIS BENSON AND JEAN GODDARD ..... 595
Evaluation of the Oxidation-Reduction Dye Test for the Determination of Virulence of Mycobacteria *in Vitro*. ROBERT A. PATNODE, CAROLYN K. WEINKLE, AND CARROLL BEASLEY ..... 599
Growth Studies on Chromogenic Acid-Fast Bacilli, with Particular Emphasis on Their Oxygen Requirements. VICTOR J. CABELLI, WILLIAM E. ELLIOT, AND ROBERT J. MCELROY ..... 604
Case Reports:
Destructive Primary Tuberculosis of the Faucial Tonsil. CHISHOLM MCDOWELL ..... 612
Transmission of Genital Tuberculosis from Husband to Wife via the Semen. JOHN K. LATTIMER, HENRY P. COLMORE, GRANT SANGER, DOUGLAS H. ROBERTSON, AND FRED C. MCLELLAN ..... 618
Acute Benign Histoplasmosis. J. ROBERT LEE AND WILLIAM M. M. KIRBY ..... 625
Editorial: The Fiftieth Anniversary of the National Tuberculosis Association. ESMOND R. LONG ..... 631

(*Continued on inside back cover*)

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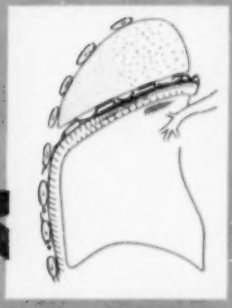

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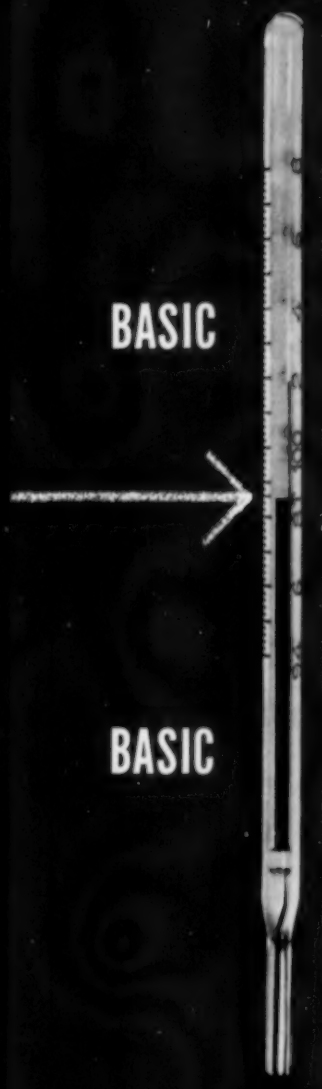


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1. Hobby, G. L., Lenert, T. F., Rivoire, Z. C., Donikian, M., and Pikula, D.: Am. Rev. Tuberc. 67:808, 1953.

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Accumulating experimental data appear to confirm the hypothesis that atherosclerosis is a metabolic disease subject to important nutritional influences. A recent study* which gives support to this concept shows that a diet moderately high in fat and high in cholesterol, but deficient in methionine and cystine, when fed to the Cebus monkey for an adequate time, produces hypercholesterolemia and atherosclerotic lesions in this animal.

The dietary regimen used in this study provided a protein in the ration deficient in the sulfur amino acids cited. This regimen increased serum cholesterol concentration to 300 to 800 mg. per cent and led to vascular lesions. These lesions "were in the ascending aorta but extended from the valves to the left ventricle to the proximal portions of the carotid and femoral arteries. Minimal lesions . . . [were] observed in the coronary arteries. The aortic lesions were . . . characterized by the presence of lipid-laden phagocytes and increase in collagen and elastic fibers. The lipids were in part cholesterol derivatives."

The study reports further that the hypercholesterolemia due to the dietary deficiency of sulfur amino acids "could be largely prevented by feeding 1 Gm. per day of dl-methionine or l-cystine as supplements to the diet. After the serum concentration had become elevated, it could be restored to normal by feeding 1 Gm. of dl-methionine . . . daily."

As the authors state, it would be both naive and premature today to attribute human atherosclerosis to deficiency of an amino acid. However, even though only foreshadowing applicability in the human realm, the findings in this study reemphasize the vital role, realized and potential, which *complete* protein plays in metabolism.

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*Mann, G. V.; Andrus, S. B.; McNally, A., and Stare, F. J.: Experimental Atherosclerosis in Cebus Monkeys, J. Exper. Med. 98:195 (Sept.) 1953.

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# THE GROWTH OF INTRACELLULAR TUBERCLE BACILLI IN RELATION TO THEIR VIRULENCE[1]

G. B. MACKANESS,[2] N. SMITH, AND A. Q. WELLS

(Received for publication December 14, 1953)

## INTRODUCTION

In studying the action of drugs upon tubercle bacilli growing within living cells (1), it was observed that only virulent strains could be relied upon to multiply rapidly and destroy the host cells within a convenient period of time. The capacity of any strain to multiply intracellularly appeared to reflect the characteristic virulence of that strain *in vivo*. As no precise method of comparing the virulence of strains of tubercle bacilli then existed, it seemed worth while to examine several strains of widely different virulence to see whether their capacity to multiply within isolated monocytes *in vitro* could be related to the growth rates of the same strains *in vivo*.

Soon after the commencement of this work, detailed studies on the multiplication *in vivo* of several strains of the tubercle bacillus were reported by Pierce, Dubos, and Schaefer (2). The present observations, although more limited, are in substantial agreement with the findings of these writers and, in addition, show a correlation between the rates of growth *in vivo* and those observed in monocyte cultures *in vitro*. It is recognized that to compare growth rates in monocytes of the rabbit *in vitro* and in organs of the mouse *in vivo* is not ideal.

## MATERIALS AND METHOD

*Cultures:* The strains of tubercle bacillus which have been used in this and accompanying studies are listed below. All strains have been maintained continuously in Tween[3]-albumin liquid medium. The virulent strains have been passaged in the guinea pig at intervals of approximately six months.

*Branch* (virulent bovine type): This strain has been extensively employed in the present study and in previous studies in this laboratory. A short description and history of the strain is appended.[4]

[1] From the Sir William Dunn School of Pathology, Oxford, England.

[2] Present address: John Curtin School of Medical Research, Australian National University, Canberra, Australia.

[3] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

[4] *M. tuberculosis, bovine type, strain Branch.* This bovine strain of tubercle bacillus was isolated post mortem from a fatal case of human tuberculosis in 1947. It was recovered on

*H37Rv* (virulent human type): A standard virulent strain obtained from the Trudeau Laboratory (Saranac Lake, New York). Virulence for the guinea pig appears to have been fully retained (3).

*H37Ra* (attenuated human strain): Obtained from the Trudeau Laboratory. The strain has been maintained in this laboratory for three years in liquid Tween-albumin medium. At the outset of these studies it gave the characteristic heaped-up colonial form, without peripheral skirting, which has been described by Middlebrook, Dubos, and Pierce (19).

*R1Rv* (attenuated human strain): Obtained from the Trudeau Laboratory (Saranac Lake, New York) (5).

*BCG* (attenuated bovine strain): The strain used in these investigations was freshly isolated for the purpose from the current vaccine strain supplied by the State Serum Institute, Copenhagen (Batch number 1043).

In experiments of the type to be described, great care must be exercised in the maintenance of the stock cultures. For the suspensions used for experimental purposes, it has been the practice to select all of the single bacilli which can be obtained from liquid cultures by differential centrifugation. To prevent dead or regressed bacilli from becoming highly concentrated in the suspensions, stock cultures must be maintained by frequent regular subculture and care taken to prevent evaporation from the medium and drying of the bacterial cells on the walls of the culture vessel. In the absence of these precautions, irregular results are often obtained.

Stock cultures were maintained by transferring 2 per cent of a seven-day growth to 5 ml. of fresh Tween-albumin medium containing 0.25 per cent bovine albumin (Armour fraction V) and 0.05 per cent Tween 80. The cultures, which were not agitated, were incubated at 37°C. in sealed, screw-capped bottles of 30 ml. capacity.

*Preparations of bacillary suspensions:* After growth for a specified time, one or more Tween-albumin cultures were gently drawn up and down in a Pasteur pipette to aid dispersion. They were then centrifuged for ten minutes (220 *g.*) to deposit all but the most highly dispersed bacilli. The supernatant was transferred to a conical tube and concentrated by recentrifugation. The deposit was resuspended in a small volume of fresh Tween-albumin medium and, after careful mixing, it was again centrifuged (three minutes at 220 *g.*). The resulting supernatant consisted very largely of single bacilli. Cultures of BCG needed a longer period of centrifugation in order to obtain a degree of dispersion similar to that of other strains. The suspensions of dispersed bacilli were counted in a Petroff-Hausser chamber under dark-ground illumination. In some cases the count was adjusted by further dilution with Tween-albumin medium.

*Preparation and maintenance of monocyte cultures:* Details of the technique have already been given (1).

Monocytes were obtained from oil-induced exudates in the peritoneal cavity of normal adult rabbits of the crossed strain bred in this laboratory. Four days after injection of the paraffin, the peritoneal cavity was washed out aseptically with saline and the yield of monocytes was concentrated and resuspended in fresh rabbit serum. The monocyte suspension was then counted in a hemocytometer. Parasitization was effected by mixing known numbers of monocytes and of bacilli. Volumes of the mixture, not exceeding 0.5 ml., were transferred to flat-bottomed bottles lined with paraffin wax and centrifuged at 850 *g.* for five minutes at room temperature. Without disturbing the deposit, the vessels were then

---

Dorset's egg medium and was subcultured on this medium for three years. For experimental purposes, it has been maintained continuously in Tween-albumin liquid medium. It has been passaged from time to time through rabbits and has remained fully virulent. Injected intravenously in rabbits, 0.01 mg., moist weight, of bacilli kills in twenty-five to thirty days. The organism grows in serpentine cords on oleic-acid–albumin agar, but after approximately ten days in Tween-albumin liquid medium all semblance of cord formation is lost and the bacilli assume a short cocco-bacillary or even coccal form. The name Branch is that of the man from whom the strain was isolated.

placed at 6°C. for one hour, at the end of which time the uptake of bacilli by the monocytes had usually proceeded to completion. The parasitized monocytes were then resuspended in chilled Hanks' solution, counted in a hemocytometer, diluted to an appropriate cell density, and introduced into the center-well culture chamber already described (1).

In the standard technique, the chambers were filled with suspensions containing $10^6$ cells per ml. The peripheral moat of the culture chamber was half filled with nutrient medium consisting of 20 per cent aged and Seitz-filtered rabbit serum in Hanks' solution, saturated with a gas mixture containing 95 per cent oxygen and 5 per cent carbon dioxide, giving a final pH of 7.2 to 7.4. No antibacterial substance was incorporated in the culture medium except when specified.

Medium was changed at twenty-four- or forty-eight-hour intervals, and the gas bubble was rotated twice a day to promote mixing between the central well and peripheral moat. Cultures were observed at frequent intervals, and at specified times one or more cultures were fixed by heat and stained in batches with Ziehl-Neelsen and methylene blue.

*Measure of bacterial growth in monocyte cultures:* Since the monocyte population does not increase by mitotic division and does not diminish significantly during long periods of incubation, except when destroyed by a rapidly expanding parasite population, it is possible to measure changes in the number of bacilli in a culture against the relatively fixed number of monocytes present. For this purpose a standard procedure was adopted. In one representative stained culture from each series, approximately 500 cells were counted along two diameters of the culture area. Each monocyte was scored for its content of bacilli. The level of infection present in a culture was expressed in the following terms.

*Average number of bacilli per infected monocyte:* This value, in conjunction with the percentage of infected monocytes, fixes with considerable precision the initial level of infection.

*Average number of bacilli per monocyte of the total population:* If the bacilli multiply and kill some of the monocytes, there is a tendency for them to become redistributed among the surviving and often uninfected cells. Hence, as a measure of the total change in size of the bacterial population, this value is the most sensitive.

*Percentage of infected monocytes:* A change in the percentage of infected monocytes indicates redistribution or digestion of bacilli. This value may change quite markedly even when few monocytes have died and extracellular multiplication of bacilli has been restrained by streptomycin. This is probably because death of the most heavily infected monocytes releases large numbers of bacilli for reingestion.

*Coefficient of increase:* This value has been used to express the change in size of the bacterial population. The coefficient of increase for a given period of incubation was taken as the ratio of the average number of bacilli per monocyte of the total population to the average number present before the onset of multiplication. The figure obtained is an accurate measure of the increase in bacterial numbers only if the number of monocytes in the culture has not changed during the period of observation. In practice, counts were made only in cultures in which detectable death of monocytes had not occurred; one or two exceptions to this rule are noted in appropriate parts of the text.

*Percentage of monocytes containing more than 20 bacilli:* It is not easy to count with accuracy more than 20 bacilli in a monocyte; such cells have therefore been grouped together and expressed as a percentage of the total population. If the percentage is high, the calculated coefficient of increase is lower than the true increase. This value also expresses crudely for any given strain the extent of bacterial multiplication but, since strains vary greatly in their cytotoxicity, comparison between strains is unreliable. Replicate counts seemed unnecessary and were seldom made; they always showed close agreement. An illustration of the agreement between replicates will be found in the third paper of this series (6).

## *Inoculation of Mice*

Swiss albino mice known to be free from bacterial infection were used. They were inoculated intravenously in a tail vein with a volume of 0.5 ml. Preliminary experiments had

shown that variation in inoculum size could be greatly reduced by increasing the volume of the inoculum from 0.2 to 0.5 ml.

*Preparation of tissue homogenates:* Mice were killed with chloroform, and organs for culture were removed aseptically to a Potter-Elvejhem homogenizer. The barrel of the homogenizer was engraved to show a volume of 10.0 ml., and 0.1 per cent bovine albumin was added to this mark. The tissue was then ground mechanically at 2,850 r.p.m. for thirty seconds. The homogenates were cell-free on microscopic examination.

*Enumeration of viable bacilli:* Viable counts were made by the method described by Fenner, Martin, and Pierce (7). Decimal dilutions of the homogenates were made in 0.1 per cent bovine albumin, and ten replicate drops of suitable dilutions were plated on oleic-acid-albumin agar. The plates were sealed and incubated for fourteen days or until satisfactory growth had been obtained.

## RESULTS

### *Observations on the Growth of Bacilli in Monocyte Cultures*

*The effect of the age of the bacillus upon its growth in an intracellular habitat:* Early in these experiments it was observed that tubercle bacilli of the same strain did not always behave in the same way. Previous studies with monocyte cultures infected with virulent bacilli had shown a remarkable consistency in the multiplication of intracellular bacilli. It soon became clear that with attenuated strains the age of the Tween-albumin culture used to infect the monocytes could greatly affect the subsequent growth rate of the bacilli. This phenomenon is most easily demonstrated with the strain R1Rv.

Suspensions of bacilli of strain R1Rv were prepared in the usual way from Tween-albumin cultures which had been incubated for one, two, and three weeks, respectively. The viability of these suspensions was determined by comparing total and viable counts. In confirmation of the findings of others (8–11), obtained either by the same method or by direct observation of microcultures, it was found that virtually all bacilli were viable in all three suspensions (table 1). On all of the plates microcolonies were detected on the eighth day and were as large and as numerous on those inoculated with three-week-old bacilli as on those inoculated with younger bacilli. Thus, the three inoculating suspensions were equivalent in terms of their capacity to grow on a complete medium.

Monocytes from a normal rabbit were infected with an approximately equal number of bacilli from one-, two- and three-week-old cultures (table 2).

In eight days, the intracellular bacilli derived from one-week-old cultures in-

TABLE 1

TOTAL AND VIABLE COUNTS ON SUSPENSIONS OF BACILLI, STRAIN R1RV, PREPARED FROM ONE, TWO, AND THREE-WEEK-OLD TWEEN-ALBUMIN CULTURES

| AGE OF CULTURE | TOTAL COUNT | VIABLE COUNT |
|---|---|---|
| *weeks* | | |
| 1 | $5.1 \times 10^7$ | $4.4 \times 10^7$ |
| 2 | $6.8 \times 10^7$ | $6.4 \times 10^7$ |
| 3 | $10.4 \times 10^7$ | $9.4 \times 10^7$ |

TABLE 2

NUMBER OF BACILLI PRESENT AFTER ONE AND EIGHT DAYS' INCUBATION IN MONOCYTE CULTURES INFECTED WITH BACILLI, STRAIN R1Rv, TAKEN FROM ONE, TWO AND THREE-WEEK-OLD TWEEN-ALBUMIN CULTURES

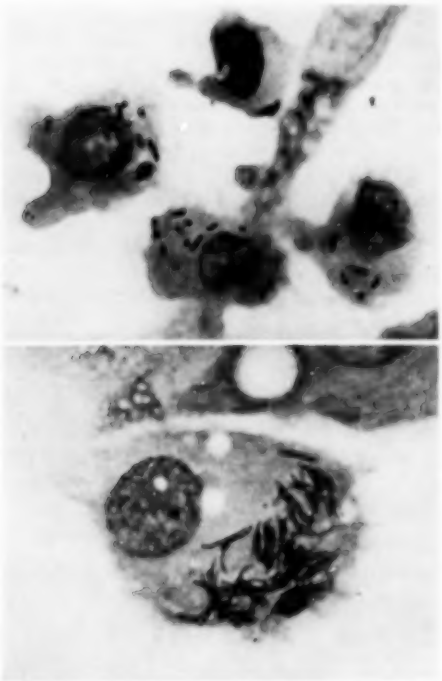
| | PERIOD OF INCUBATION OF MONOCYTE CULTURE | | | | | |
|---|---|---|---|---|---|---|
| | 1 day | | | 8 days | | |
| | AGE OF INFECTING SUSPENSION (WEEKS) | | | | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Average number of bacilli per infected monocyte | 2.3 | 3.1 | 3.2 | 5.6 | 4.1 | 3.3 |
| Average number of bacilli per monocyte of the total population | 1.2 | 1.7 | 2.0 | 3.3 | 2.7 | 2.0 |
| Percentage of monocytes infected | 52 | 57 | 63 | 59 | 66 | 62 |
| Percentage of monocytes containing more than 20 bacilli | 0 | 0 | 0 | 6.1 | 2.9 | 0.3 |
| Coefficient of increase | | | | 2.8 | 1.6 | 1.0 |

creased almost 300 per cent; those from two-week-old cultures by only 60 per cent; while bacilli from three-week-old cultures had not begun to multiply.

The morphology of the actively multiplying bacilli in one-week-old cultures on the eighth day is seen to be quite different from that of the unregenerated bacilli of three-week-old cultures (figures 1 and 2). In monocytes infected with two-week-old bacilli, elongated growing forms and short unregenerated bacilli were both seen. The bacilli from three-week-old cultures finally began to elongate on approximately the eleventh day of incubation.

The retarded growth of aged bacilli in an intracellular environment appears to be related to the virulence of the strain. Aged bacilli of the virulent bovine strain Branch have never failed to multiply in monocytes within the first forty-eight hours of incubation, while bacilli of the virulent human strain H37Rv of equivalent age may show a latency of three or four days before the onset of multiplication. On the other hand, with BCG, inocula prepared from cultures more than seven to ten days old have never been observed to multiply intracellularly in the life of a monocyte culture (usually about four weeks). The results with strain R1Rv given above fall between these two extremes.

*Relative growth rates of different strains of the tubercle bacillus in isolated monocytes in vitro:* The first planned experiment to compare the intracellular growth rates of different strains of bacilli was performed in parallel *in vitro* and *in vivo*. The suspensions of bacilli were prepared from seven-day-old cultures in Tween-albumin medium. The results of the *in vivo* experiment are recorded later. Observations in monocyte cultures were unsatisfactory, for the inocula used were too large to permit a quantitative measure of the relative growth rates. Qualitatively, however, the same pattern was observed as on subsequent occasions. Cultures infected with H37Rv died on the third day of incubation. Those in-



FIGS. 1 and 2. Stained monocyte cultures showing the difference in appearance after eight days' incubation of tubercle bacilli, strain R1Rv, in monocytes infected with bacilli from three-week-old and one-week-old Tween-albumin cultures. (Ziehl-Neelsen-methylene blue, ×1,400).

FIG. 1 (Upper). From culture infected with "three-week-old" bacilli. The unregenerated bacilli are short and thick.

FIG. 2 (Lower). From culture infected with "one-week-old" bacilli. The actively multiplying bacilli are long and slender. The monocyte appears larger than in figure 1 because of more complete removal of culture medium at the time of fixation.

fected with the strains R1Rv and H37Ra died on the fifth day; while those infected with the strain BCG were still alive on the seventh day.

In all subsequent experiments, monocytes were infected with smaller numbers of bacilli. Under these conditions, monocytes infected with virulent bacilli will tolerate greater numbers of bacilli acquired slowly as a result of multiplication. In a typical experiment, using bacilli from seven-day-old cultures, the change in size of the bacterial population of each strain was determined after three, five, seven, and nine days. The results are recorded in tables 3 and 4. The differences between strains are apparent at each interval during incubation.

The results for strain BCG are incomplete owing to contamination of the cultures, but many other experiments have established the characteristic behavior of this strain. Even young bacilli of proved viability, and in large numbers, do not begin to multiply in less than ten to twelve days.

TABLE 3

NUMBERS OF BACILLI PRESENT IN MONOCYTE CULTURES INFECTED WITH YOUNG BACILLI OF FOUR STRAINS OF MYCOBACTERIUM TUBERCULOSIS AT INTERVALS DURING INCUBATION

| | STRAIN | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H37Rv | | | | | R1Rv | | | | | H37Ra | | | | | BCG | | | | |
| | Duration of Incubation, Days | | | | | | | | | | | | | | | | | | | |
| | 1 | 3 | 5 | 7 | 9 | 1 | 3 | 5 | 7 | 9 | 1 | 3 | 5 | 7 | 9 | 1 | 3 | 5 | 7 | 9 |
| Average number of bacilli per infected monocyte | 2.6 | 7.3 | 11.9 | >20* | Overgrown | 3.1 | 4.6 | 5.5 | 7.0 | 13.2 | 4.9 | 4.7 | 5.0 | 7.1 | 10.8 | 4.9 | 4.1 | 3.7 | — | — |
| Average number of bacilli per monocyte of total population | 1.2 | 3.2 | 6.8 | >20 | | 1.5 | 2.4 | 2.9 | 4.1 | 9.4 | 2.5 | 2.3 | 2.6 | 4.0 | 8.2 | 2.5 | 2.0 | 1.5 | — | — |
| Percentage of monocytes infected | 45 | 45 | 58 | 100 | | 49 | 52 | 53 | 58 | 71 | 50 | 48 | 53 | 57 | 71 | 50 | 49 | 47 | — | — |
| Percentage of monocytes containing more than 20 bacilli | 0 | 3.2 | 29 | 100 | | 0 | 0.4 | 2.4 | 3.8 | 29 | 0.4 | 0.9 | 1.0 | 1.8 | 13 | 0.3 | 0.4 | 0 | — | — |

* Death of monocytes had already occurred in this culture.

TABLE 4

COEFFICIENTS OF INCREASE FOR YOUNG TUBERCLE BACILLI OF FOUR STRAINS GROWING IN MONOCYTES AT 37°C.

Calculated from the Data of Table 3

| | DURATION OF INCUBATION, DAYS | | | |
|---|---|---|---|---|
| | 3 | 5 | 7 | 9 |
| H37Rv | 2.7 | 5.7 | >20 | complete overgrowth |
| R1Rv | 1.6 | 1.9 | 2.7 | 6.3 |
| H37Ra | 0.9 | 1.0 | 1.8 | 3.3 |
| BCG | 0.8 | 0.6 | — | — |

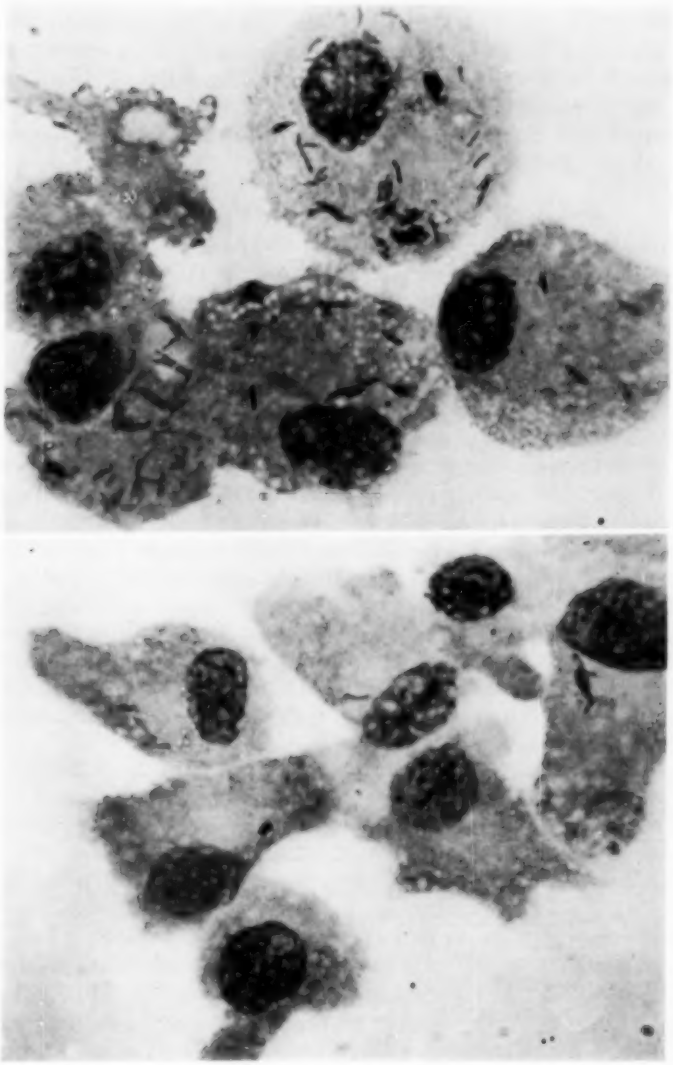
It was not expected that strain H37Ra would multiply intracellularly. This finding is discussed later.

As an illustration of the marked difference in the growth rates of virulent and attenuated strains, figures 3 and 4 show the appearance, when fixed and stained after five days' incubation, of cultures infected with strains H37Rv and BCG. At this time 40 per cent of the inoculated bacilli of strain BCG had disappeared. Some may have failed to stain, but it is more likely that they had been completely destroyed since some of the monocytes contained golden-brown granular debris. Destruction of BCG with the formation of pigment has been observed in tissue culture by Wermel (12) and Jonescu and Jonescu (13).

It may be seen from table 3 that apparent differences in growth rates of the various strains are due largely to differences in lag periods. Unfortunately, the data are not exact or extensive enough to permit a calculation of true growth rates during the phase of active multiplication of intracellular bacilli. It is therefore unknown whether strains growing intracellularly differ in their true growth rates. The impression is gained that attenuated strains never multiply as rapidly as virulent strains.

In table 3 it may be seen that no significant redistribution took place in R1Rv cultures between the third and seventh days of incubation, confirming the visual impression that no significant death of monocytes had occurred in this interval. On this basis it is possible to calculate the relative increase in the bacterial population for the two-day intervals, three to five and five to seven days; these were a 1.2- and 1.4-fold increase, respectively. For the strain H37Rv, on the other hand, there was a 2.7-fold increase in the two-day interval one to three days; and, in view of the large number of cells which contained more than 20 bacilli on the fifth day of incubation, the increase was perhaps even higher in the interval three to five days.

Findings similar to those described have been obtained in four recent experiments. In one of these experiments, ingested bacilli were demonstrated to be viable by transferring monocyte cultures to oleic-acid–albumin agar after twenty-four hours' incubation. The method has been described (16). In the case of each strain, colonies grew from more than 90 per cent of the infected monocyte resi-



FIGS. 3 and 4. Stained monocyte cultures showing the difference in the extent of bacterial multiplication after five days' incubation in cultures infected with strains H37Rv (upper) and BCG (lower). (Ziehl-Neelsen-methylene blue, ×1,400)

dues. In many other experiments, some of which are referred to in accompanying papers (6, 14), intracellular growth rates of two or more strains have been compared under a variety of conditions. In all cases the relative growth rates of the several strains have remained in the order H37Rv, R1Rv, H37Ra, and BCG. In two earlier experiments, strain H37Ra was at the bottom of this series; at the time it showed no evidence of multiplication in an intracellular environment. This important discrepancy is discussed below.

### *Observations in Vivo*

*Preliminary experiments:* In order to demonstrate that the method used for tissue homogenation did not damage bacilli present in the tissues, a suspension of highly dispersed bacilli (strain R1Rv) was added to an homogenate of whole spleen. After mixing, a sample was taken for serial dilution and plating. The homogenate was then ground for a second time in the manner described above. The viable counts obtained on the splenic homogenate before and after grinding were $3.80 \times 10^6$ and $3.75 \times 10^6$, respectively.

Preliminary experiments were also performed to find the most suitable organ and interval of time in which to study the relative growth rates of different strains *in vivo*. Twenty-four mice were injected intravenously with 0.2 ml. of a suspension of R1Rv prepared from a seven-day culture in Tween-albumin medium. In this preliminary experiment viable counts were performed only on the lungs and spleens of animals killed at intervals. The results are presented in table 5. They show considerable variation, which was attributed to a failure to deliver intravenously a constant volume of inoculum. In subsequent experiments, a larger inoculating volume of 0.5 ml. was used, and much of the variation due to this error was eliminated. In agreement with the findings of Pierce, Dubos, and Schaefer (2), the bacterial population in the spleen appeared to expand for only

TABLE 5

NUMBER OF VIABLE UNITS OF TUBERCLE BACILLI, STRAIN R1Rv, IN THE LUNGS AND SPLEENS OF 4 MICE AT INTERVALS AFTER INTRAVENOUS INOCULATION*

| | Day | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | | 8 | | 12 | | 16 | |
| | Organ | | | | | | | | | | | |
| | S | L | S | L | S | L | S | L | S | L | S | L |
| 1 | 152† | 2.7 | 220 | 0.8 | 1,845 | 8.0 | — | — | 2,905 | 52 | 256 | 225 |
| 2 | 51 | 3.3 | 820 | 1.6 | 270 | 2.0 | 1,150 | 4.5 | 1,400 | 11 | 3,875 | 95 |
| 3 | 21 | 0.1 | 484 | 1.9 | 1,065 | — | 350 | 1.0 | 1,000 | 6 | 324 | 41 |
| 4 | 180 | 2.8 | 381 | 2.7 | 705 | 2.0 | 915 | 3.0 | — | — | 980 | 107 |
| Average | 101 | 2.2 | 476 | 1.8 | 1,121 | 4.0 | 805 | 2.8 | 1,768 | 23 | 1,360 | 117 |

* Inoculum contained $9.7 \times 10^5$ viable units

† Figures multiplied by $10^2$ give the number of viable units per organ

S = spleen L = liver

TABLE 6

DISTRIBUTION OF BACILLI IN THE ORGANS OF MICE FOLLOWING INTRAVENOUS INOCULATION OF A DISPERSED SUSPENSION OF BCG*

| | TIME | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 Minutes | | | 4 Hours | | | 24 Hours | | |
| | Mouse Number | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Spleen | 141† | 225 | — | 840 | — | 530 | 230 | 850 | 485 |
| Liver | 290 | 915 | 760 | 640 | 575 | 900 | — | 1,000 | — |
| Lung | 53 | 12 | — | 16 | — | 4 | 0.4 | 0.6 | 0.9 |
| Total | 484 | 1,152 | — | 1,496 | — | 1,434 | — | 1,850 | — |

* Inoculum contained $2.1 \times 10^6$ viable units

† The figures when multiplied by $10^3$ give the number of viable units per organ

a limited period of time. Very few bacilli were present in the lungs, and multiplication could not be detected before the twelfth day. In this experiment, however, only a fraction of the total inoculum had been accounted for. The liver was therefore included in a further preliminary experiment.

Nine mice were injected intravenously with 0.5 ml. of a suspension containing $2.1 \times 10^6$ viable units. The bacillary content of lungs, spleen, and liver was determined in groups of three mice at intervals of five minutes, four hours, and twenty-four hours. The results, as presented in table 6, indicate that at the end of twenty-four hours virtually the whole of the inoculum had become fixed in liver and spleen. It was decided to ignore the lungs and to obtain baseline counts on the initial content of bacilli in both liver and spleen at the end of twenty-four hours.

*Relative growth rates of four strains of the tubercle bacillus in the livers and spleens of mice:* Four groups, each of 12 mice, were injected intravenously with 0.5 ml. of a suspension of highly dispersed bacilli prepared from seven-day-old Tween-albumin cultures, using respectively the four strains, H37Rv, R1Rv, BCG, or H37Ra. By direct counts each suspension was estimated to contain $1.0 \times 10^6$ bacterial units per ml.

At the end of twenty-four hours, half of each group of mice were killed and the content of viable bacterial units in liver and spleen was determined. After eight days, the rest were killed and treated in the same way (table 7). The coefficients of increase for each strain were calculated from the averages obtained for each organ (table 8). In all instances growth was much more rapid in the spleen than in the liver.

The order of the relative rates of increase of the strains is the same as that found *in vitro*. In both cases, strains H37Rv and BCG occupy the two extremes, while strains R1Rv and H37Ra, which do not differ greatly from each other, occupy an intermediate position.

Pierce, Dubos, and Schaefer (2) found that their strain of H37Ra did not mul-

tiply in the tissues of the mouse, a finding which justifies the use of the term "avirulent." Since the strain used in the present experiments not only multiplied, but did so more rapidly than BCG, it had presumably undergone a change in virulence during the long period of cultivation in liquid medium. Workers at the Rockefeller Institute have found that their strain of H37Ra, when propagated in liquid media, now and then produces variants with the serpentine growth-pattern characteristic of more virulent strains (15). Although the strain used in these experiments still shows no evidence of cord formation when growing on oleic-acid–albumin agar, it must be presumed to differ from the classical avirulent strain. This is particularly likely since it is known from earlier experience that two years ago the parent strain was incapable of growth, and rapidly became nonviable, in isolated monocyte cultures (16).

## DISCUSSION

Attenuated strains of the tubercle bacillus have long been known to multiply *in vivo*, but it has only recently been established by Pierce, Dubos, and Schaefer (2) that the initial rate and the extent of this multiplication in the tissues of the

TABLE 7

NUMBER OF VIABLE UNITS OF TUBERCLE BACILLI, STRAINS H37Rv, R1Rv, BCG, H37Ra, IN THE LIVERS AND SPLEENS OF MICE AT ONE AND EIGHT DAYS AFTER INTRAVENOUS INOCULATION

| STRAIN | MOUSE NUMBER | | | | | | AVERAGE | MOUSE NUMBER | | | | | | AVERAGE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | |
| H37Rv | | | | | | | | | | | | | | |
| Spleen | 91* | 30 | 152 | 93 | — | — | 92 | 20,000 | 8,000 | 10,000 | 16,000 | 18,000 | 12,000 | 14,000 |
| Liver | 430 | 515 | 475 | 520 | — | — | 485 | 11,000 | 14,000 | 33,000 | 10,000 | 13,000 | 17,000 | 16,000 |
| R1Rv | | | | | | | | | | | | | | |
| Spleen | 55 | 65 | 63 | 61 | 54 | 64 | 60 | 1,215 | 960 | 960 | 1,750 | 1,470 | — | 1,269 |
| Liver | 555 | 380 | 380 | 370 | 470 | 455 | 435 | 960 | 1,130 | 690 | 1,500 | 650 | — | 984 |
| H37Ra | | | | | | | | | | | | | | |
| Spleen | 29 | 41 | 46 | 46 | 77 | 73 | 52 | 630 | 910 | 805 | 1,115 | — | 880 | 868 |
| Liver | 425 | 415 | 380 | 390 | 390 | 560 | 423 | 1,320 | 635 | 460 | 970 | 640 | 490 | 751 |
| BCG | | | | | | | | | | | | | | |
| Spleen | 55 | 195 | 64 | 145 | 105 | 80 | 102 | 120 | 235 | — | 630 | 415 | — | 350 |
| Liver | 220 | 230 | 240 | 250 | 380 | 30 | 270 | — | — | 285 | 285 | 975 | 300 | 461 |

* Figures when multiplied by $10^2$ give the number of bacillary units per organ.

TABLE 8

COEFFICIENTS OF INCREASE FOR BACILLI OF FOUR STRAINS DURING GROWTH FOR EIGHT DAYS IN THE LIVER AND SPLEEN OF MICE

Calculated from the Figures in Table 7

| STRAIN | SPLEEN | LIVER |
|---|---|---|
| H37Rv | 152 | 33 |
| R1Rv | 21 | 2.3 |
| H37Ra | 17 | 1.8 |
| BCG | 3.4 | 1.7 |

mouse are correlated with the virulence of the bacilli. These writers appear to show that for a given inoculum the initial rate of multiplication determines the maximum number of bacilli eventually reached in the tissues.

Except for a varying capacity to grow in the form of serpentine cords, characteristic differences between strains have not been revealed in ordinary cultures. It is common experience that mammalian strains of the tubercle bacillus grown in a liquid medium to which they have become adapted do not differ conspicuously in their growth characteristics, although they may differ widely in their pathogenicity for a susceptible host. Fenner and Leach (9) found almost identical growth constants for H37Rv and BCG and very little difference between the growth constants of young and old bacilli of the same strain.

When growing in the isolated monocyte, on the other hand, there are marked differences between strains, particularly in the duration of the lag period and the susceptibility of the bacilli to digestion by the monocytes. The more attenuated the strain, the longer apparently was the lag period, and the same was true with increasing age of the culture used for parasitizing the monocytes. In certain strains, Branch and R1Rv, the beginning of growth can always be recognized by the change from short cocco-bacillary to elongated forms. By watching the process of elongation, it was possible to see that protracted lag periods result from a failure to grow and not from coincident multiplication and destruction of bacilli.

The effect of age of the inoculum on the lag period of intracellular bacilli in monocyte cultures appears to have its counterpart *in vivo*. Bloch (17) observed that aged bacilli are significantly less pathogenic for the mouse and rabbit than young bacilli; Fenner and Leach (9) have confirmed this observation. The effect might well be accounted for by the protracted lag period of aged bacilli in an intracellular environment. The enormously protracted lag period of aged bacilli of the strain BCG inside cells is associated with greatly diminished viability, which may explain the reduction in the number of lesions found by Fenner and Leach (9) in mice infected with old inocula of this strain.

When tubercle bacilli are introduced into the tissues, they are soon taken up by the mononuclear cells. For this reason the behavior of bacilli of a particular strain in monocyte cultures *in vitro* may well reflect their behavior in the early parasitic existence *in vivo*. The present data, by showing a parallel between growth rate *in vivo* and in cells *in vitro*, appear to support this view. It is possible, therefore, that virulence of the tubercle bacillus should be looked upon as a function of capacity to survive and multiply in an intracellular environment.

The work of Suter (18) on tubercle bacilli growing *in vitro* within cells has led him to the conclusion that virulent and attenuated strains grow equally well in monocytes *in vitro*, although he could find no evidence that an avirulent strain would grow. Certain aspects of Suter's technique are, however, open to criticism. Any quantitative study of the relationships between host cell and parasite must take account, not only of any change in the parasite population, but also any change in the host cells. The number of bacilli contained in the host cells may be altered by multiplication or destruction of the host cells as well as of the bacilli.

For instance, when host cells are destroyed by growing bacilli, the liberated organisms may disappear from the preparation or be ingested by surviving cells. An examination of Suter's published reports gives reasons for believing that large numbers of monocytes were lost or destroyed in his preparations. Figures 1 and 5 in his paper show almost total replacement of monocytes by proliferating spindle cells, which have the morphology of fibroblasts or serosal cells. These could not have arisen by transformation of monocytes but must have come from multiplication of parent cells present in the original exudate.

The disappearance of the monocytes in Suter's cultures, and their replacement by proliferating spindle cells, makes it difficult to assess the significance of his bacterial counts. As Suter pointed out, virulent bacilli kill monocytes much more rapidly than do attenuated bacilli. The loss of monocytes in Suter's cultures may well have been more rapid when virulent bacilli were tested, and this would tend to obscure any difference between the growth rates of virulent and attenuated strains when measured by changes in the distribution of bacilli within the cells. It is believed that this difficulty was almost completely avoided in the present experiments by frequently inspecting the cultures to see that all of the cells were alive and making the final measurements of bacterial growth before any observable destruction of monocytes had occurred. Destruction too slight to be observed was nonetheless revealed by an increase in the percentage of monocytes infected.

Under the present experimental conditions, and presumably also *in vivo*, tubercle bacilli show evidence of delayed adaptation to an intracellular environment. This becomes more marked in proportion to the degree of attenuation. At present it is not clear whether the inhibitory effect of an intracellular environment is due to a lack of adaptation of the parasite to the nutritional conditions inside the cell or whether it derives from an active inhibition of growth by some product of the host cell's metabolic activity.

## SUMMARY

The relative growth rates of four strains of tubercle bacilli, strains H37Rv, R1Rv, BCG, and H37Ra, have been measured quantitatively in rabbit monocyte cultures *in vitro* and in the livers and spleens of mice *in vivo*. The growth rates of different strains varied greatly, and a correlation was found between the relative rates of increase in the two situations. It is suggested that the virulence of tubercle bacilli may be a function of capacity to survive and multiply in an intracellular environment.

It was also shown that the physiologic state of bacilli affects the rate of adaptation of intracellular bacilli. Aged bacilli have a longer lag period than young bacilli. The effect of age on the intracellular growth pattern increases markedly with the degree of attenuation. Thus, aged bacilli of a virulent bovine type show only a small increase in lag period; whereas, in an inoculum of BCG of equivalent age, most of the bacilli are virtually incapable of growth and are largely digested intracellularly *in vitro*.

SUMARIO

*El Crecimiento de los Bacilos Tuberculosos Intracelulares en Relación con Su Virulencia*

Los relativos coeficientes de desarrollo de cuatro cepas de bacilos tuberculosos, H37Rv, R1Rv, BCG y H37Ra, fueron computados cuantitativamente en cultivos en monocitos de conejo *in vitro* y en los hígados y bazos de ratones *in vivo*. Los coeficientes de las distintas cepas variaron considerablemente, notándose correlación entre los coeficientes relativos de aumento en las dos situaciones. Sugiérese que la virulencia de los bacilos tuberculosos puede ser una función de la capacidad para sobrevivir y multiplicarse en un ambiente intracelular.

Demostróse además que el estado fisiológico de los bacilos afecta el índice de adapatación de los bacilos intracelulares. Los bacilos viejos muestran un período más largo de retardo que los jóvenes. El efecto de la edad sobre la proliferación intracelular aumenta notablemente conforme al grado de atenuación. Los bacilos viejos de tipo bovino virulento no muestran más que un pequeño aumento del período de retardo, en tanto que, en inóculo de BCG de edad semejante, la mayor parte de los bacilos son virtualmente incapaces de desarrollarse, siendo en gran parte digeridos intracelularmente *in vitro*.

RESUME

*La croissance des bacilles tuberculeux intracellulaires par rapport à leur virulence*

Les vitesses relatives du développemente de quatre souches de bacilles tuberculeux, souches H37Rv, R1Rv, BCG et H37Ra ont été mesurées quantitativement, *in vitro*, dans des cultures de monocytes de lapin et, *in vivo*, dans le foie et la rate de la souris. La vitesse de développement des diverses souches variait considérablement et, dans ces deux conditions, une corrélation a été observée entre les vitesses relatives de l'accroissement. L'auteur propose de considérer la virulence du bacille de Koch comme pouvant être une fonction de la capacité de survivre et de se reproduire dans un miiieu intracellulaire.

Il a été également démontré que l'état physiologique des bacilles affecte la vitesse d'adaptation du bacille intracellulaire. Les bacilles âgés ont une période d'adaptation de plus grande durée que celle des bacilles jeunes. L'effet de l'âge sur les modalités du développement intracellulaire augmente considérablement avec le degré de l'atténuation. C'est ainsi que la phase du développement lent n'est que peu allongée pour les bacilles bovins virulents, tandis qu'avec l'inoculum de BCG, d'âge équivalent, la plupart des bacilles sont virtuellement incapables de se développer et subissent la digestion intracellulaire *in vitro*.

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# THE GROWTH OF TUBERCLE BACILLI IN MONOCYTES FROM NORMAL AND VACCINATED RABBITS[1]

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## INTRODUCTION

Humoral mechanisms have not been shown conclusively to play a part in the immunity of animals to tuberculosis. Instead there has arisen a concept of "cellular immunity," supported by experimental findings (1). In an accompanying paper (2), evidence is presented that the virulence of tubercle bacilli is related to their capacity to survive and multiply in the cytoplasm of isolated monocytes *in vitro*. The same experimental method has been applied to a study of the growth of tubercle bacilli in monocytes from normal and vaccinated rabbits to determine whether those of immunized animals are intrinsically altered in such a way as to alter the growth characteristics of bacilli which have been ingested by them.

## MATERIALS AND METHOD

The strains of tubercle bacillus used, the method for parasitization, preparation, and maintenance of rabbit monocyte cultures, and the procedure for measuring the relative growth rates of intracellular tubercle bacilli are the same as those used in the first paper of this series (2).

### *Immunization*

Immunity to the tubercle bacillus in most experimental systems of host and parasite is only relative and usually quite tenuous. For this reason immunity in the monocyte, if it exists, is unlikely to be pronounced and would manifest itself only under the most advantageous conditions. The rabbit is highly resistant to infection with virulent human-type tubercle bacilli. But, as Lurie (3) has shown, bacilli of this type multiply rapidly in the rabbit during the early stages of the infection. This suggests that the immunity of the rabbit to human-type bacilli is of an acquired type. It was believed that host cells derived from rabbits successfully combating a virulent human-type infection would be ideal material with which to detect an immune mechanism in the monocyte. Most of the studies reported in this paper have been done with monocytes from rabbits vaccinated intravenously with large inocula of the strain H37Rv. Rabbits vaccinated with BCG have also been used.

Several dosage ranges were used for vaccination but, in all of the experiments to be described, nine-month-old cross-bred rabbits were injected intravenously with 0.2 ml. of a seven-day Tween-albumin culture of H37Rv (approximately $10^8$ viable units); this dose was repeated three months later. BCG-vaccinated animals were injected intravenously

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with a similar dose of a seven-day Tween-albumin culture. Vaccinated animals were used for monocyte production in the weeks following their second vaccinating injection.

Tuberculin was injected intradermally over the cartilage at the base of the ear. All vaccinated animals were sensitive to tuberculin (OT 1:100, 0.1 ml.) at the time of injecting liquid paraffin into the peritoneal cavity for the production of a mononuclear cell exudate. In each experiment, two series of monocyte cultures were prepared in parallel; one with monocytes from a vaccinated rabbit, the other from a normal tuberculin-negative control.

## EXPERIMENTAL RESULTS

### *Rabbits Immunized with H37Rv*

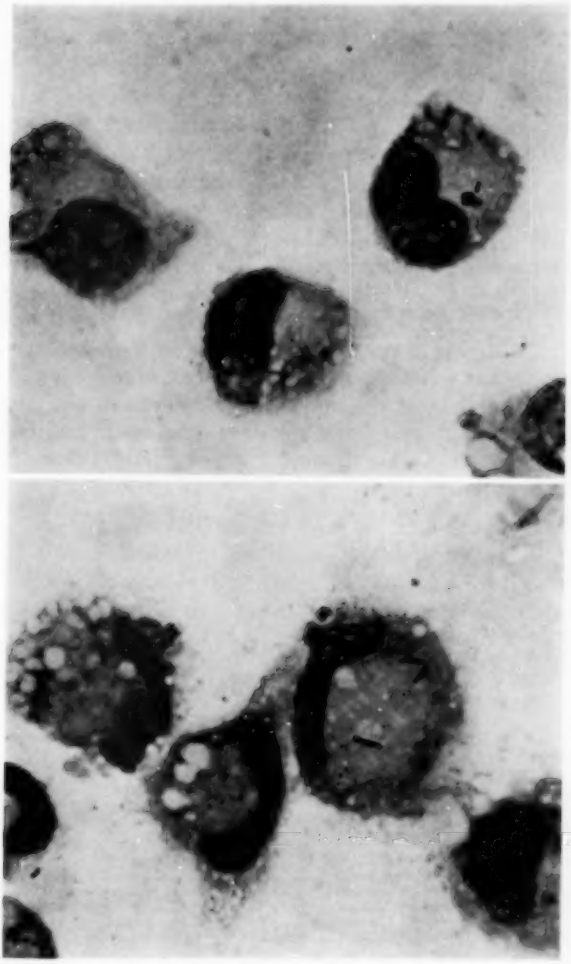
*Lag period and multiplication rate of virulent bacilli in normal and "immune" monocytes:* Bacilli of two of the strains used in these experiments, the bovine-type Branch and the human-type R1Rv, undergo marked changes in morphology during their growth cycle in Tween-albumin medium; both become much shorter with increasing age of the culture. The change occurs more rapidly, and is more extreme, in the Branch strain. Fenner and Leach (4) have shown a small increase in length of bacilli of strains H37Rv and BCG in the early stages of growth in Tween-albumin medium. The changes in length undergone by bacilli of the strains Branch and R1Rv are so marked, however, that they can be used to give an indication of the duration of the lag phase of intracellular bacilli. In the preceding paper (2) it was shown that aged bacilli are slower to adapt themselves to an intracellular environment than are young bacilli. It might be expected, therefore, that aged bacilli would show to best advantage the influence of an immune mechanism in the host cell.

Monocytes were obtained from a normal, tuberculin-negative rabbit and from a vaccinated rabbit twenty-eight days following the second vaccinating dose of H37Rv. The normal and "immune" monocytes were infected with equivalent numbers of bacilli from a three-week-old Tween-albumin culture of the strain Branch. Streptomycin in a concentration of 3.0 $\gamma$ per ml. of culture medium was used to restrain the growth of extracellular bacilli; at this concentration it has little effect upon the growth of intracellular bacilli (5). Cultures were fixed at six-hour intervals for the first twenty-four hours and at twelve-hour intervals thereafter.

There was no detectable change in the length of bacilli until the end of twenty-four hours. At this time, occasional bacilli in both normal and "immune" monocytes showed well-marked elongation. At thirty-six hours, virtually all bacilli in the monocytes of both normal and vaccinated animals had become grossly elongated. The lag periods in the two situations appeared, therefore, to be identical. The process of regeneration of bacilli in monocytes from the vaccinated animal is shown in figures 1 and 2.

Following the phase of elongation, the bacterial cells began to multiply. The numbers of bacilli present in the cultures at twenty-four hours, before the onset of multiplication, and after three days' incubation are set out in table 1. The rates of increase in normal and "immune" monocytes did not appear significantly different.

This experiment was repeated on two occasions, and again the lag periods and



FIGS. 1 and 2. Stained preparations of cultures of monocytes from an immunized rabbit showing the early phase of bacillary elongation in "immune" monocytes infected with virulent tubercle bacilli of bovine type, strain Branch. (Ziehl-Neelsen-methylene blue, ×1,400)

FIG. 1. (Upper). Monocytes containing cocco-bacillary forms six hours after the commencement of incubation.

FIG. 2. (Lower). Monocytes containing elongated bacilli thirty-six hours after the commencement of incubation.

TABLE 1

NUMBERS OF BACILLI, STRAIN BRANCH, PRESENT AFTER ONE AND THREE DAYS' INCUBATION IN CULTURES OF MONOCYTES FROM NORMAL AND H37RV-IMMUNIZED RABBITS

| | PERIOD OF INCUBATION OF MONOCYTE CULTURE, DAYS | | | |
|---|---|---|---|---|
| | 1 | | 3 | |
| | Source of Monocytes | | | |
| | Normal Rabbit | Immunized Rabbit | Normal Rabbit | Immunized Rabbit |
| Average number of bacilli per infected monocyte | 4.5 | 5.3 | 7.9 | 8.6 |
| Average number of bacilli per monocyte of total population | 2.2 | 2.7 | 5.3 | 5.8 |
| Percentage of monocytes infected | 49 | 51 | 67 | 68 |
| Percentage of monocytes containing more than 20 bacilli | 0.4 | 0 | 9.8 | 4.9 |
| Coefficient of increase | | | 2.4 | 2.2 |

growth rates of bacilli were similar in the monocytes from normal and vaccinated animals. In one of these experiments, the density of monocytes in the culture chambers was higher than in the standard procedure (× 1.5) and a longer interval was left between changes of medium (three days). Both of these changes in procedure tend to diminish the rate of increase in bacterial numbers and will be discussed in a subsequent communication. The results of this experiment are recorded in table 2.

TABLE 2

NUMBERS OF BACILLI, STRAIN BRANCH, PRESENT AFTER TWO, FOUR, AND SIX DAYS' INCUBATION IN CULTURES OF MONOCYTES FROM NORMAL AND H37RV-IMMUNIZED RABBITS

| | PERIOD OF INCUBATION OF MONOCYTE CULTURE, DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 2 | | 4 | | 6 | |
| | Source of Monocytes | | | | | |
| | Normal Rabbit | Immunized Rabbit | Normal Rabbit | Immunized Rabbit | Normal Rabbit | Immunized Rabbit |
| Average number of bacilli per infected monocyte | 3.7 | 3.6 | 5.6 | 5.1 | 9.9 | 9.2 |
| Average number of bacilli per monocyte of total population | 2.5 | 2.3 | 4.4 | 4.1 | 8.4 | 7.8 |
| Percentage of monocytes infected | 66 | 64 | 79 | 79 | 85 | 84 |
| Percentage of monocytes containing more than 20 bacilli | 0 | 0 | 1.4 | 2.1 | 9.4 | 8.0 |
| Coefficient of increase | | | 1.8 | 1.8 | 3.4 | 3.4 |

*Multiplication of attenuated bacilli in normal and "immune" monocytes:* Three unsuccessful attempts were made to compare the lag periods of regressed bacilli of the attenuated strain R1Rv in normal and "immune" cells.

The lag period inside living monocytes for aged bacilli of this strain may be as long as fourteen days (2). It is rarely possible to keep two series of monocyte cultures comparable in all respects for this length of time. One or other series of cultures was always found to contain fibroblasts in sufficient numbers to permit their rapid growth. In this event, deterioration of the monocyte population invariably occurred and comparative studies became impossible. The same difficulty was encountered with the strain BCG, for even young bacilli of this strain have a greatly protracted lag period within monocytes.

Young bacilli of the strain R1Rv grow with sufficient rapidity in the monocyte to permit a comparative study of intracellular growth rates.

Monocytes were obtained from a normal tuberculin-negative rabbit and from an immunized rabbit thirty-five days following the second vaccinating dose of H37Rv. Normal and "immune" monocytes were infected with equivalent numbers of bacilli from a seven-day-old Tween-albumin culture of strain R1Rv. Streptomycin at a concentration of 3.0 $\gamma$ per ml. was incorporated in the culture medium. Cultures were fixed at one, five, and eight days, and counts were made on the number of bacilli present. The results are recorded in table 3.

Although the counts shown in table 3 show that redistribution of bacilli had taken place during incubation, it was unassociated with any appreciable monocyte death. It appears to have been similar in extent in both series of cultures. In this and in a similar experiment, no significant difference was found between growth rates in normal and "immune" monocytes.

TABLE 3

NUMBERS OF BACILLI, STRAIN R1Rv, PRESENT AFTER ONE, FIVE, AND EIGHT DAYS' INCUBATION IN CULTURES OF MONOCYTES FROM NORMAL AND H37Rv-IMMUNIZED RABBITS

| | PERIOD OF INCUBATION OF MONOCYTE CULTURE, DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 5 | | 8 | |
| | Source of Monocytes | | | | | |
| | Normal Rabbit | Immunized Rabbit | Normal Rabbit | Immunized Rabbit | Normal Rabbit | Immunized Rabbit |
| Average number of bacilli per infected monocyte | 6.3 | 4.6 | 7.5 | 6.3 | 13.6 | 11.6 |
| Average number of bacilli per monocyte of total population | 4.3 | 3.0 | 6.4 | 4.8 | 12.6 | 10.7 |
| Percentage of monocytes infected | 68 | 65 | 75 | 72 | 93 | 93 |
| Percentage of monocytes containing more than 20 bacilli | 0.5 | 0.7 | 5.7 | 5.1 | 30 | 26 |
| Coefficient of increase | | | 1.5 | 1.6 | 2.9 | 3.6 |

TABLE 4

NUMBERS OF BACILLI, STRAIN BRANCH, PRESENT AFTER ONE AND THREE DAYS' INCUBATION IN CULTURES OF MONOCYTES FROM NORMAL AND BCG-IMMUNIZED RABBITS

| | PERIOD OF INCUBATION OF MONOCYTE CULTURE, DAYS | | | |
|---|---|---|---|---|
| | 1 | | 3 | |
| | Source of Monocytes | | | |
| | Normal Rabbit | Immunized Rabbit | Normal Rabbit | Immunized Rabbit |
| Average number of bacilli per infected monocyte | 6.7 | 6.6 | 17 | 15 |
| Average number of bacilli per monocyte of total population | 5.6 | 5.1 | 16 | 14 |
| Percentage of monocytes infected | 84 | 78 | 95 | 94 |
| Percentage of monocytes containing more than 20 bacilli | 3.1 | 4.1 | 55 | 45 |
| Coefficient of increase | | | 2.9 | 2.8 |

*Rabbits Immunized with BCG*

Two experiments were performed with monocytes from rabbits immunized with BCG. In one, performed twenty-one days after vaccination, normal and "immune" monocytes were infected with bacilli from seven-day-old Tween-albumin cultures of the two strains, H37Rv and Branch. The numbers of bacilli present initially were comparable in the two series of cultures but were a little too high to permit satisfactory estimates of the rates of increase. All cultures died on the fourth day of incubation.

In the second experiment, performed twenty-eight days after vaccination, monocytes were infected with bacilli from a three-week-old culture of the Branch strain. Streptomycin was absent from the medium. Bacterial counts at one and three days are presented in table 4. The rate of increase in bacterial numbers was the same in both series of cultures.

*Survival Time of Normal and "Immune" Monocytes Infected with Virulent Tubercle Bacilli*

If a sufficient number of virulent bacilli are ingested by a normal monocyte *in vitro*, it dies immediately. When smaller numbers of bacilli (5 to 10 per cell) are ingested, death is usually delayed until the onset of growth (5). If very few bacilli have been ingested, there may be a long delay before death, and living, motile monocytes containing large numbers of bacilli may be present in the cultures. It thus appears that the tolerance of the monocyte for virulent bacilli is greater when the bacilli are acquired slowly by a process of multiplication within the cell. It was thought that, if immune cells had any specific capacity to inhibit the growth of bacilli, this might be demonstrated by comparing the survival time of cultures of normal and "immune" cells. It was found, however, that at all levels of infection "immune" monocyte cultures always died sooner

than those of normal cells if the two had been infected with equivalent numbers of bacilli. The following experiment provides an illustration.

Normal and "immune" monocytes were infected with bacilli of the two strains, Branch and H37Rv. The number of bacilli present initially in the cultures were as follows:

| | BRANCH | | H37Rv | |
|---|---|---|---|---|
| | Normal | "Immune" | Normal | "Immune" |
| Average number of bacilli per infected monocyte | 5.3 | 4.8 | 5.2 | 4.8 |
| Average number of bacilli per monocyte of the total population | 2.4 | 2.1 | 3.1 | 2.1 |
| Percentage of infected monocytes | 44 | 46 | 60 | 49 |
| Percentage of monocytes containing more than 20 bacilli | 1.4 | 1.4 | 0.7 | 1.7 |

In each case a smaller number of bacilli were present in the "immune" cultures. For four days all cultures remained in perfect condition. Between the fourth and fifth days, monocytes in both sets of "immune" cultures died rapidly. In cultures of normal cells death occurred more gradually, signs of degeneration being first detected at the end of five days; destruction was not complete until the end of the sixth day of incubation.

In this experiment, half of the cultures of each series contained isoniazid (1.0 $\gamma$ per ml. of culture medium), which prevents multiplication of intracellular bacilli (6). All of these survived beyond the tenth day of incubation.

Premature death of monocytes from vaccinated rabbits has been observed in almost all experiments in the present series, although the difference in survival time was never marked. The phenomenon may be an expression of cellular hypersensitivity, in which case it appears to be dependent upon the synthesis of antigen by living and multiplying bacilli, for it was suppressed by exposing the cultures to isoniazid.

## DISCUSSION

The results which have just been described do not encourage the belief that the monocytes of vaccinated rabbits have any specific capacity to suppress the growth of tubercle bacilli resident within them. The rates of increase of bacterial populations in normal and "immune" monocytes scarcely differed. The time taken for elongation to occur in regressed bacilli was the same in monocytes from normal and vaccinated rabbits. The latter finding seems convincing evidence that the biosynthetic activities of the tubercle bacillus are not specifically interrupted within the phagocytic cells of vaccinated animals.

Lurie (7) reached an opposite conclusion. He infected mononuclear cells from normal and vaccinated rabbits with virulent human-type tubercle bacilli and implanted them in the anterior chamber of rabbits' eyes, "immune" cells being placed in one eye, normal cells in the other. When material from each eye was examined culturally and histologically fourteen to twenty days after implanta-

tion, growth of bacilli was found to have been markedly retarded in the eye implanted with "immune" cells. It is doubtful whether this justifies Lurie's conclusion that "Active tuberculosis confers on the mononuclear phagocytes themselves increased bacteriostatic properties for the tubercle bacillus which are independent of the immune body fluids or of the organ environment in which they grow." Not only was the number of bacilli in the two eyes different, but so was the nature of the lesion. If Lurie's figures 5 and 6 are compared, it will be seen that in one, the normal eye, there was a loose semi-necrotic reaction of mixed cells; in the other, the "immune" eye, a compact cellular lesion similar in nature to a consolidated epithelioid tubercle had developed. If these are representative of the tissue reactions which developed, there may be an alternative explanation of Lurie's experimental findings. Chase has succeeded in transferring tuberculin hypersensitivity by means of exudative mononuclear cells (8), and this has been repeatedly confirmed (9). It is possible, therefore, that local hypersensitivity was transferred to the eye implanted with cells from the immunized animal; and there are good reasons for believing that hypersensitivity, induced in this way, could remain confined to the one eye. The present writer is not yet fully aware of the implications of this; some, at least, of the conditions which prevail in the lesion of reinfection might be supposed to have existed here. Among these conditions is the undisputed phenomenon of accelerated tubercle formation. The mononuclear cells of the lesion observed in the "immune" eye were possibly a part of this reaction even though they contained some of the carbon introduced in the implanted cells.

A more direct method of approach to the settlement of this question seems necessary. Lurie quotes the work of Kallós in support of his conclusions. Kallós (10) mixed virulent bacilli with exudative mononuclear cells in fresh plasma and allowed the plasma to clot in Carrel flasks. In the presence of either monocytes or plasma derived from allergic guinea pigs, Kallós found that "multiplication of tubercle bacilli may perhaps have been slightly retarded." When monocytes and plasma both came from allergic animals, no evidence of bacterial multiplication could be found. Initially the bacilli in Kallós' experiments were not intracellular and were present in unknown numbers; it is difficult, therefore, to know what factors were operating in this experimental system. His finding in the preparations with both "immune" cells and "immune" plasma is important and requires confirmation. Except for the short-term experiments of Hanks and Evans (11), in which serum-cell mixtures had no effect upon the viability of tubercle bacilli *in vitro*, and the experiments of Suter (12), to be discussed below, this is the only reported observation upon the behavior of tubercle bacilli in a system in which living host cells were available for the ingestion of bacilli which had been exposed to circulating immune bodies.

Brieger, Fell, and Smith (13) were unable to distinguish between the rates of growth of virulent bacilli in tissue cultures made from normal rabbit spleen infected *in vitro* and in those made from spleens of tuberculous animals explanted one to seventeen days after intravenous or intrasplenic inoculation. Growth appeared more rapidly in explants removed from the body six to seventeen days

following infection than in explants removed from newly infected animals one to six days following infection. In both cases, bacilli were equally few in the mononuclear cells which emigrated from the tissue explants. If this finding is confirmed, it would follow that no substantial immunity existed in the host cells at any time during the first seventeen days of a virulent infection; but in Brieger, Fell, and Smith's paper there is a lack of precise information concerning the numbers of bacilli present in the explants.

Suter (12) has recently published what appears to be unequivocal evidence of antiblastic immunity in the monocytes of vaccinated rabbits and guinea pigs. The method used in his studies was criticized in the preceding paper of this series (2) on the general ground that the number of monocytes present in the cultures changed greatly during the period of observation, so that no reliable assessment could have been made of changes in the number of intracellular bacilli. In the present context, the further complication of cellular hypersensitivity arises. If, as is suggested by the present study, allergically sensitized monocytes are destroyed somewhat more rapidly than normal monocytes by antigen liberated from proliferating bacilli, heavily parasitized monocytes would be destroyed in "immune" cultures more rapily than in normal cultures. The data of the present experiments show, however, that the percentage of monocytes containing more than 20 bacilli was never greatly different in cultures from normal and immunized rabbits, so that premature death of "immune" monocytes did not occur to any significant extent in the period during which bacterial counts were made. Cellular hypersensitivity does not appear, therefore, to have affected the results obtained in the present study. This is perhaps attributable to the fact that the rabbit, in contrast to the guinea pig, develops only a mild degree of tuberculin hypersensitivity.

The evidence so far adduced for the existence of antiblastic immunity in the mononuclear phagocytes of vaccinated or tuberculous animals would not appear to be conclusive; and the results of the present experiments have failed to reveal any detectable antituberculous activity in the monocytes of rabbits vaccinated with virulent or attenuated strains of the tubercle bacillus.


## SUMMARY

The growth rates of both virulent and attenuated tubercle bacilli have been compared quantitatively in cultures of monocytes obtained from normal and vaccinated rabbits. The lag period, as determined by the time taken for aged bacilli to change from coccobaciilary to elongated forms, and the subsequent growth rates of intracellular bacilli were found to be identical in monocytes from normal rabbits and from rabbits vaccinated with bacilli of strains H37Rv or BCG.

## SUMARIO

*El Crecimiento de Bacilos Tuberculosos en Monocitos de Conejos Normales y Vacunados*

Compañáronse cuantivamente las tasas del crecimiento de bacilos tuberculosos tanto virulentos como atenuados en cultivos de monocitos obtenidos de conejos normales y

vacunados. El período de retardo, determinado por el tiempo consumido por los bacilos viejos para pasar de formas cocobacilares a alargadas y las subsiguientes tasas del crecimiento de bacilos intracelulares resultaron idénticos en los monocitos procedentes de conejos normales y de conejos vacunados con bacilos de las cepas H37Rv o BCG.

RESUME

*La croissance des bacilles tuberculeux dans les monocytes de lapins vaccinés ou non vaccinés*

Les vitesses de développement de bacilles tuberculeux virulents ou de virulence atténuée ont été comparées quantitativement dans les cultures de monocytes prélevés chez des lapins vaccinés et non vaccinés. La durée du retard, déterminée par le temps nécessaire au bacille âgé pour passer de la forme coccobacillaire à la forme allongée, et les vitesses de développement ultérieurs des bacilles intracellulaires ont été trouvées identiques dans les monocytes des lapins non vaccinés et dans ceux des lapins vaccinés avec les bacilles des souches H37Rv ou BCG.

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# CORRELATION OF BIOLOGIC PROPERTIES OF STRAINS OF MYCOBACTERIUM WITH THEIR INFRARED SPECTRUMS[1,2]

## III. Differentiation of Bovine and Human Varieties of *M. Tuberculosis* by Means of Their Infrared Spectrums

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### INTRODUCTION

Three years ago there was presented in this journal the first report of this work, which in its most general aspect has been to see to what extent the infrared spectrums of complex mixtures could be useful as a control on fractionations of biologic materials as well as a means of detecting known or suspected biologic differences. The investigations began in 1948, and the complex mixtures were primarily those found in the tubercle bacillus. It was noted in an earlier report (1) that it was necessary to standardize all procedures in the growth and fractionation of cultures if reproducible and, hence, useful spectrums were to be obtained. This technique was then applied to show that it was possible to demonstrate chemical differences between the H37Rv and H37Ra strains of the tubercle bacillus (2).

In continuing this work it seemed of interest to apply the combination of techniques of fractionation and spectroscopy to other problems of the same general nature. This paper presents a comparison of the infrared spectrums of fractions prepared from human and bovine tubercle bacilli.

### MATERIALS AND METHODS

The cultures used for this investigation were all recent isolates obtained from the following sources. Culture No. 251 (human) was obtained from Dr. Charlotte Colwell of the Veterans Administration Hospital, Hines, Illinois. Cultures Nos. 274 and 275 (bovine) were obtained from the U. S. Department of Agriculture through the courtesy of Dr. H. Schoening. Inocula containing 0.001 mg. (dry weight) of the bovine strains produced disease and death in rabbits in three or four weeks when injected intravenously, whereas 0.01 mg. of the human strain produced only minor lesions. However, this same human culture (No. 251) in a dilution containing $10^{-4}$ mg. produced sufficient disease in guinea pigs in six weeks to be assigned a Feldman Index (3) of 49 as an average for 5 animals.

The procedures for growth of cultures, harvesting, and alcohol treatment of the harvested cell mass are the same as were reported earlier (2). The fractionation techniques were basically the same and are summarized in the flow diagram (figure 1). This system of fractionation was improved upon by subjecting the extracts, prepared by cooling the methanol-chloroform solution to 5°C. and −30°C., to further separations on chromatographic columns or by a technique of batch adsorption and elution.

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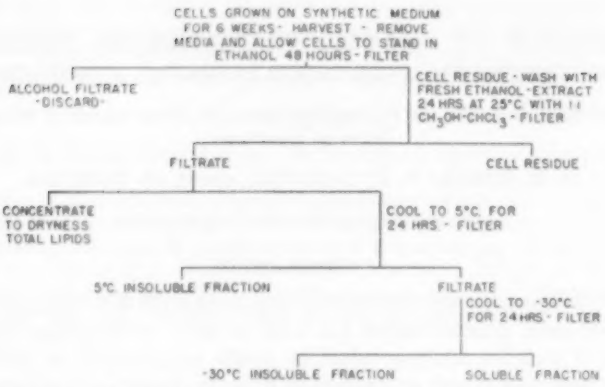
CELLS GROWN ON SYNTHETIC MEDIUM FOR 6 WEEKS - HARVEST - REMOVE MEDIA AND ALLOW CELLS TO STAND IN ETHANOL 48 HOURS - FILTER
ALCOHOL FILTRATE -DISCARD-
CELL RESIDUE - WASH WITH FRESH ETHANOL - EXTRACT 24 HRS. AT 25°C. WITH 1:1 $CH_3OH$-$CHCl_3$ - FILTER
FILTRATE
CELL RESIDUE
CONCENTRATE TO DRYNESS TOTAL LIPIDS
COOL TO 5°C. FOR 24 HRS. - FILTER
5°C. INSOLUBLE FRACTION
FILTRATE
COOL TO -30°C. FOR 24 HRS. - FILTER
-30°C. INSOLUBLE FRACTION
SOLUBLE FRACTION

FIG. 1. Flow diagram for a separation of the lipids of tubercle bacilli

The chromatographic columns (13 x 750 mm.) were packed with 30 gm. of a mixture containing one part Magnesol[4] and 2 parts Celite.[5] The fraction to be chromatographed was dissolved in petroleum ether (60° to 70°C. boiling), the insoluble residue was separated by centrifugation, and the solution was applied to the column which had previously been wet with petroleum ether. The column was developed sequentially with 200 ml. amounts of petroleum ether, petroleum ether-benzene (equal parts), benzene, benzene-ether, and ether, collecting 15 ml. amounts of the eluate, which were then dried and prepared for spectroscopy. For batch adsorption, the lipid complex was dissolved in petroleum ether, and the insoluble residue was separated. The solution was diluted so that one part of fraction was dissolved in 400 parts solvent, and was then shaken for four hours with 100 parts Magnesol. The supernatant was filtered off and collected as the petroleum ether eluate. The adsorbent was washed with fresh petroleum ether, dried, and the next solvent was introduced. The solvent series was the same as for the chromatographic separation. The filtrates form the eluates which were subsequently dried under vacuum and prepared for spectroscopic examination.

## RESULTS

It may be seen in figure 2 that there are essentially no spectral differences between the whole cells derived from a bovine culture and cells from a human culture. The spectrums of the total methanol-chloroform–soluble lipids are dissimilar, but since this fraction is not reproducible the differences are not helpful.

The first two spectrums of figure 3 were prepared from the fraction of human and bovine cultures soluble in 1:1 methanol-chloroform at 25°C. but insoluble at 5°C. This fraction is easily reproduced, but there are no significant spectral differences between similar fractions from the two varieties represented. In the early phase of this work, investigations included avian strains of tubercle bacilli. It was found that the chemical differences were so great between the avian strains and either human or bovine strains that they could be detected in the 5°C. insoluble fraction. Therefore, although this 5°C. fraction does not permit the dis-

[4] Magnesol was obtained from the Westvaco Chemical Co., South Charleston, West Virginia.

[5] Celite was obtained from the Johns Manville Co. (Filter Aid).

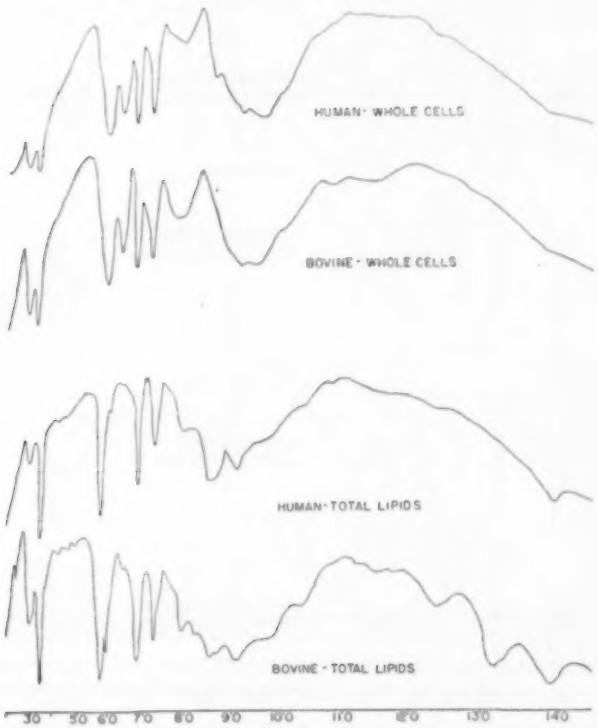


FIG. 2. Spectrums prepared from whole cells and total lipids derived from a typical human and bovine culture.

tinction between human and bovine varieties, it does serve to differentiate the avian strain from either of the former.

The last two spectrums on figure 3 are of the fraction which precipitates at −30°C. from the filtrate of the 5°C. insoluble fraction. This fraction is not as easily reproduced as the previous one; hence, some of the variations are not significant. However, a weak band appears at 6.63$\mu$ in the fraction of bovine cells which is absent in the same fraction prepared from human cells. This weak band has been shown to be present in 14 spectrums prepared from 5 bovine strains and is absent in 28 spectrums of 18 human strains. On the basis of this observation, quantities of the −30°C. fraction were prepared and subjected to further separations by column chromatography and batch adsorption.

The results of these separations are shown in figure 4. The top spectrum is the ether eluate separated by batch adsorption technique from the −30°C. insoluble fraction of the human culture No. 251. The last two spectrums are the comparable fractions prepared from bovine cultures Nos. 274 and 275. It can be seen that the absorption at 6.63$\mu$ has been markedly increased for the two bovine

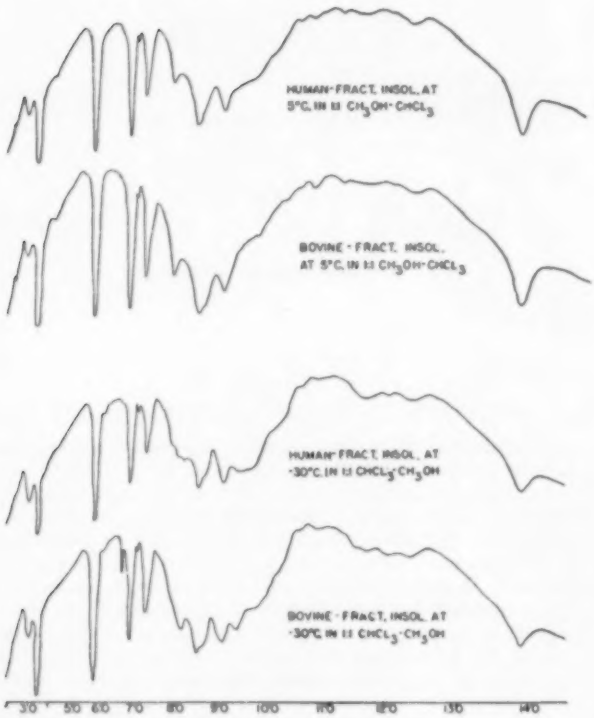
HUMAN - FRACT. INSOL. AT 5°C. IN 1:1 $CH_3OH$-$CHCl_3$
BOVINE - FRACT. INSOL. AT 5°C. IN 1:1 $CH_3OH$-$CHCl_3$
HUMAN - FRACT. INSOL. AT -30°C. IN 1:1 $CHCl_3$-$CH_3OH$
BOVINE - FRACT. INSOL. AT -30°C. IN 1:1 $CHCl_3$-$CH_3OH$
30 50 60 70 80 90 100 110 120 130 140

FIG. 3. Spectrums of the 5°C. and the −30°C. insoluble fractions prepared from a typical human and bovine culture.

fractions, whereas the band does not occur in the comparable human fraction. The benzene-ether eluate gives substantially the same result. Other obvious spectral differences exist between the fractions prepared from the two types of organisms.

Using techniques of chromatography or batch adsorption, it has been possible thus far to isolate six times from a total of 3 bovine strains a fraction spectroscopically identical to the one obtained from bovine strain No. 274.

By the same techniques, it has not been possible to isolate the component having an absorption at 6.63$\mu$ from a total of 9 recently isolated human strains.

## DISCUSSION

It appears from the results presented that it has been possible to concentrate a chemical component which may be characteristic for bovine tubercle bacilli. The presence of such a substance was first detected in a crude fraction; subsequently, as a result of the combination of chromatography and infrared spectroscopy, it was possible to concentrate this component materially. Other workers, Noll and Bloch (4), during the progress of this work, have found this combina-

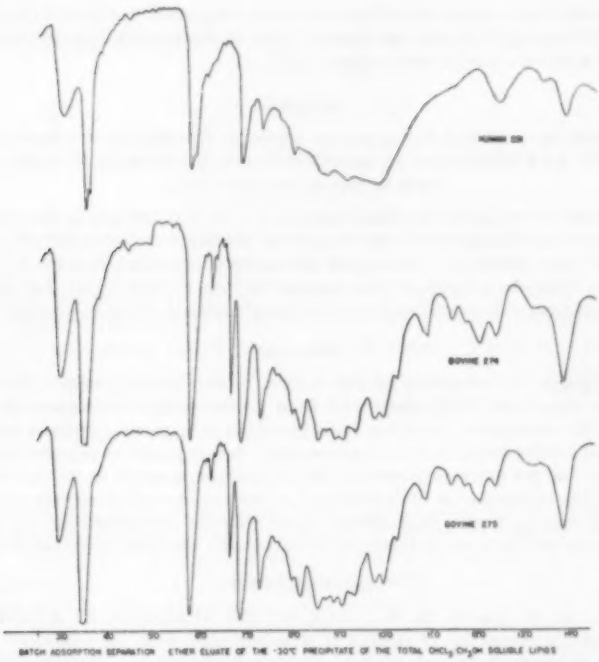


FIG. 4. Spectrums of the ether eluates separated by batch adsorption from the −30°C. insoluble fraction of strains Nos. 251 (human), 274, and 275 (bovine).

tion of techniques of value in the isolation of a toxic lipid component of tubercle bacilli.

An investigation is being made of the biologic properties of the component characteristic for bovine tubercle bacilli which is soluble in chloroform, ether, benzene, and acetone. The component has a melting range of 32° to 34°C.


## SUMMARY

A fraction has been concentrated from bovine tubercle bacilli by chromatography which is spectroscopically and, therefore, chemically distinct from any fraction thus far obtained from human tubercle bacilli. This fraction has been isolated from a total of 3 bovine strains and has been found to be absent in comparable fractions of 9 strains recently isolated from the sputum of tuberculous patients.

## SUMARIO

*Correlación de las Propiedades Biológicas de Cepas del Mycobacterium con Sus Espectros Infrarrojos: III. La Diferenciación de las Variedades Bovinas y Humanas del M. Tuberculosis por Medio de Sus Espectros Infrarrojos*

Por medio de la cromatografía se ha concentrado una fracción de bacilos tuberculosos bovinos, que es, espectroscópicamente, y por lo tanto, químicamente, distinta de toda

fracción obtenida hasta ahora de bacilos tuberculosos humanos. Esa fracción ha sido aislada de un total de tres cepas bovinas, sin que se haya encontrado en fracciones comparables de nueve cepas aisladas recientemente del esputo.

RESUME

*Corrélation entre les propriétés biologiques de souches de Mycobacterium et leur spectre infra-rouge: III. La différenciation des variétés bovines et humaines du M. tuberculosis à l'aide de leur spectre infra-rouge*

Une fraction de bacilles tuberculeux humains a été concentrée par la chromatographie; du point de vue spectroscopique et par conséquent chimique cette fraction est distincte de toute autre obtenue jusqu'à présent à partir de bacilles tuberculeux humains. Cette fraction particulière a été isolée à partir de trois souches bovines; elle manquait dans des fractions comparables obtenues dans neuf souches récemment isolées à partir de crachats.

*Addendum*

Since this paper was submitted for publication, further investigations of the conditions necessary to extract the 6.63$\mu$ component from bovine strains have given the following results: (*1*) The absorption band has been identified in extracts prepared directly from ethanol-killed bovine cultures when benzene-ether (equal parts) or petroleum ether-ether (equal parts) was the extracting solvent. (*2*) It has been possible to demonstrate the absorption band in extracts of as little as 0.1 gm. of culture (growth from four egg slant cultures). (*3*) The culture may be heat-killed without effecting the extraction. (*4*) The absorption band can be seen in benzene-ether extracts after only one hour contact with the culture.

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# THE EFFECT OF CORTISONE ON BCG INFECTION IN THE GUINEA PIG[1]

KONRAD BIRKHAUG

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## INTRODUCTION

It is well known that cortisone reduces the inflammatory reaction in tuberculosis and may result in the exacerbation of tuberculous lesions (1, 2). It therefore seemed important to determine the influence of cortisone on the response of guinea pigs to the BCG strain of tubercle bacilli. In the present study this has been determined by comparing the effects on the inflammatory response, allergic sensitization, the 'accelerated reaction' (Koch phenomenon), intradermal spread of vital dye, hematologic reactions, the evolution of BCG tuberculous lesions, and the immunity response to a challenge infection.

## MATERIALS AND METHODS

Sixty albino tuberculin-negative guinea pigs, each weighing 488 gm. plus-minus 35.7 gm., received intramuscularly in the left thigh 10 mg. of freshly prepared BCG vaccine (Lot B-139), which yielded 36 living elements (colonies) in $10^{-6}$ dilution on Löwenstein egg medium. A similar group of 60 animals, each weighing 486 gm. plus-minus 38.9 gm., received the same dose of BCG killed by heating at 70°C. for thirty minutes. Twenty-four animals in each group received intramuscularly, on alternate days for three months, 6.25 mg. of cortisone acetate[2] in 0.25 ml. of suspending agent consisting of 0.9 per cent sodium chloride, 1.5 per cent benzyl alcohol, 0.4 per cent polyoxyethylene Sorbitan monooleate, 0.5 per cent sodium carboxymethylcellulose, and sufficient distilled water to make 100 per cent. For control purposes, 12 other animals in both groups received intramuscularly 0.25 ml. of suspending agent during the same period. The remaining 24 animals in both groups received no treatment. Inoculations of cortisone and suspending agent were not made in the left thigh, where the BCG inoculum invariably produced a cold abscess with either living or killed vaccine.

## EXPERIMENTAL RESULTS

*Tuberculin sensitization:* Tuberculin sensitization was determined monthly with gradually decreasing intracutaneous doses of Old Tuberculin (OT). Data on induration measured at forty-eight hours after inoculation are presented in table 1. The tissue response to 10 mg. of OT one month after vaccination was significantly less in the cortisone-treated animals than in those receiving the suspending agent or in the untreated controls. The degree of sensitization was generally greater in the animals inoculated with living than with killed BCG. Subsequent tests with 1.0 mg. of OT in two months, and with 0.1 mg. of OT in three months, after vaccination showed the same significant difference. Although the tuberculin reaction was never fully extinguished in the cortisone-treated animals,

[1] From the Division of Laboratories and Research, New York State Department of Health, Albany, New York.

[2] Cortisone acetate kindly supplied by Merck & Co., Inc., Rahway, New Jersey.

TABLE 1

EFFECT OF CORTISONE ON THE DEVELOPMENT OF TUBERCULIN SENSITIZATION IN GUINEA PIGS INOCULATED WITH BCG VACCINE

(Twelve Animals in Each Group)

| MONTHS AFTER BCG INOCULATION | DOSE OF OLD TUBERCULIN | LIVING BCG VACCINE | | |
|---|---|---|---|---|
| | | Cortisone Group | Suspensoid Group | Untreated Group |
| | | Tuberculin Induration Area (Mm.²) | | |
| | mg. | | | |
| 1 | 10.0 | 722 ± 182 | 1,216 ± 459* | 1,517 ± 348* |
| 2 | 1.0 | 88 ± 41 | 389 ± 169* | 408 ± 216* |
| 3 | 0.1 | 48 ± 38 | 350 ± 147* | 275 ± 177* |
| | | KILLED BCG VACCINE | | |
| 1 | 10.0 | 329 ± 213 | 1,048 ± 713* | 1,385 ± 286* |
| 2 | 1.0 | 104 ± 50 | 423 ± 185* | 480 ± 154* |
| 3 | 0.1 | 24 ± 13 | 307 ± 154* | 240 ± 167* |

* Significantly different from the cortisone group at $P \leq 0.01$ level. In Fisher's 1938 tables for distribution of $t$ (quotient expressing the deviation as a multiple of its probable error), it is observed that a $P \leq 0.01$ (probability that the observed mean deviation bearing this or smaller values cannot have occurred by chance alone) requires that $t \geq 2.819$ when each group contains 12 animals or samples.

its inflammatory character was strikingly less intense than that in the controls, which showed marked induration and edema with well-defined edges.

*'Accelerated skin reaction' (Koch phenomenon)*: The 'accelerated skin reaction' in BCG multiple punctures cannot be evoked in animals and in man (3–7) in less than two to three weeks after the primary vaccination, and usually simultaneous with the development of tuberculin hypersensitiveness. When revaccination takes place at this time, the local BCG papules develop between the third and sixth days, with manifestations resembling the Koch phenomenon. This so-called 'Diagnostic BCG-Test' (6) was performed in the animals one month after vaccination by making two skin punctures with a 26-gauge needle point through several drops of 20 mg. of BCG vaccine per ml. applied to the shaved ventral surface. The maximum diameters of inflammatory reaction, as well as the thickness of the skin, measured three days later, are given in table 2.

A striking difference in inflammatory reaction was observed between the cortisone-treated and the control animals. In the former the BCG papular reaction was faintly pink and scarcely raised above the skin surface, while in the latter it was deeply reddish-brown, markedly indurated, and presented a suppurating elevated center. The papular reaction nearly healed in the cortisone-treated animals within one week, while it persisted for approximately one month in the control animals and healed in six to eight weeks after inoculation. The regional axillary and inguinal lymph nodes examined at weekly intervals up to two months after inoculation were palpably larger and firmer in the controls than in the hormone-treated animals.

TABLE 2

EFFECT OF CORTISONE ON THE ACCELERATED REACTION (KOCH PHENOMENON) IN SKIN OF GUINEA PIGS SENSITIZED WITH BCG VACCINE ONE MONTH EARLIER

(Six Animals in Each Group)

| HOURS AFTER PUNCTURE | TYPE OF BCG USED | CORTISONE GROUP | SUSPENSOID GROUP | UNTREATED GROUP |
|---|---|---|---|---|
| | | Maximum Induration (Mm.²) Across 20 Mg. per Ml. BCG Puncture | | |
| 72 | Living | 1.8 ± 0.26 | 13.0 ± 3.06* | 19.1 ± 4.24* |
| 72 | Killed | 1.7 ± 0.37 | 10.1 ± 0.58* | 14.8 ± 4.50* |

* Significantly different from the cortisone group at $P \leq 0.01$ level.

TABLE 3

EFFECT OF CORTISONE ON INTRACUTANEOUS SPREAD OF VITAL DYE IN GUINEA PIGS SENSITIZED WITH BCG VACCINE

(Twelve Animals in Each Group)

| MONTHS AFTER BCG INOCULATION | HOURS AFTER DYE INJECTION | LIVING BCG VACCINE | | |
|---|---|---|---|---|
| | | Cortisone Group | Suspensoid Group | Untreated Group |
| | | Spread (Mm.²) of 0.025 Ml. Pontamine Sky 2 Per Cent Dye | | |
| 2 | 1 | 46 ± 10 | 254 ± 40* | 261 ± 121* |
| 2 | 4 | 120 ± 31 | 442 ± 87* | 407 ± 154* |
| 2 | 24 | 314 ± 143 | 1,182 ± 260* | 1,005 ± 114* |
| | | KILLED BCG VACCINE | | |
| 2 | 1 | 37 ± 10 | 260 ± 83* | 281 ± 99* |
| 2 | 4 | 56 ± 16 | 415 ± 85* | 406 ± 123* |
| 2 | 24 | 217 ± 95 | 1,196 ± 230* | 1,033 ± 127* |

* Significantly different from the cortisone group at $P \leq 0.01$ level.

*Intradermal spread of vital dye:* The present writer and associates have demonstrated (8, 9) that sensitization of guinea pigs with BCG evokes a restricting influence on the spread of a nonspecific vital dye (Pontamine Sky Blue) in the skin. The same procedure was used to determine the effect of cortisone treatment. In table 3 are presented the diagonal measurements of colored skin after one, four, and twenty-four hours following the intradermal injection of 0.025 ml. of a 2.16 per cent solution of the dye. The spread of dye was significantly less in the cortisone-treated animals than in the controls; no difference existed between the spread of dye in the animals vaccinated with living or killed BCG.

*Hematologic changes:* Sabin and her associates stated (10–12) that "the blood picture in tuberculosis can hardly be said to be pathognomonic, but should be of considerable aid in diagnosis. However, its greatest service is in prognosis, since the quantitative abnormal changes in the cells are progressive with advancing disease." Blood studies in experimental tuberculosis by the present writer and Schjelderup (13–14) confirmed this statement.

Accordingly, the *intra vitam* effects of prolonged cortisone treatment on the

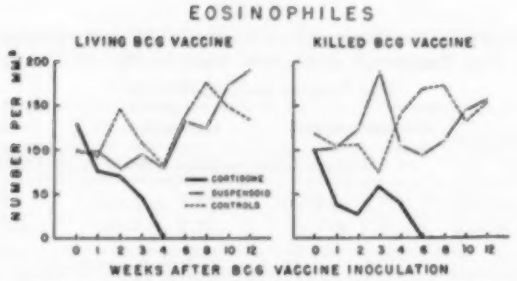


FIG. 1. Weekly deviations in circulating eosinophiles in guinea pigs immunized with living or killed BCG and treated with cortisone.

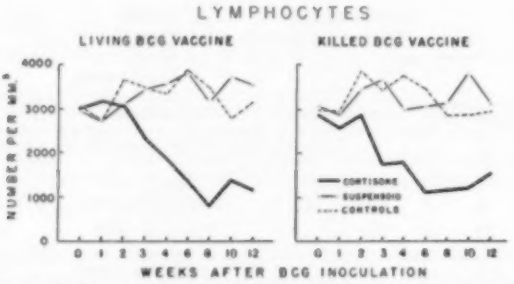


FIG. 2. Weekly deviations in circulating lymphocytes in guinea pigs immunized with living or killed BCG and treated with cortisone.

hemogram of animals inoculated with living or with killed BCG were determined. No significant changes were observed in hemoglobin, erythrocyte sedimentation rate, and leukocyte counts at weekly intervals during the three postvaccination months. The absolute number of circulating eosinophiles, however, was significantly depressed after one week of hormone treatment, and this cell disappeared completely from circulation after four weeks of treatment (figure 1). The absolute number of circulating lymphocytes diminished significantly in the hormone-treated animals (figure 2); the absolute number of banded or segmented leukocytes remained within bounds of biologic variation (figure 3). The absolute number of circulating monocytes (figure 4) and the monocyte-lymphocyte ratio (M/L) (figure 5) significantly increased in the hormone-treated animals after one month of treatment in comparison with the controls. The latter showed the usual delayed increments in these cells and ratios following inoculation with living or killed BCG. It appears, therefore, that the above modifications in the hemogram are accelerated by cortisone treatment.

*Lymphonodular and visceral changes:* Cortisone treatment was discontinued after three months when each animal had received approximately 300 mg. Twelve animals in each of the 6 groups were killed to determine quantitatively whether the treatment had affected the localized tuberculous lesions normally

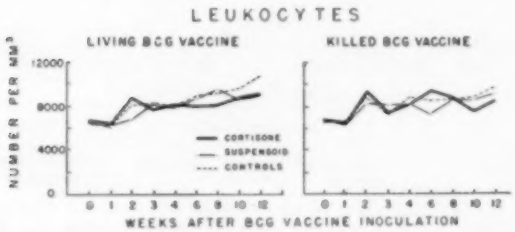


FIG. 3. Weekly deviations in total circulating leukocytes in guinea pigs immunized with living or killed BCG and treated with cortisone.

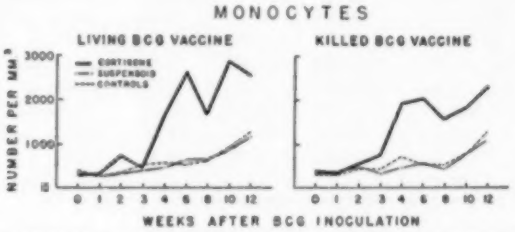


FIG. 4. Weekly deviations in circulating monocytes in guinea pigs immunized with living or killed BCG and treated with cortisone.

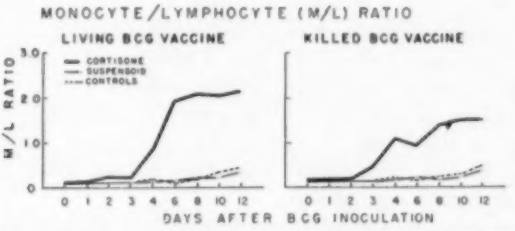


FIG. 5. Weekly deviations in the monocyte/lymphocyte ratio in guinea pigs immunized with living or killed BCG and treated with cortisone.

produced by 10 mg. of living or killed BCG. The post-mortem data on the weights of lymph nodes, adrenals, spleens, livers, and the lungs of 72 animals are presented in table 4. The average body weight of the cortisone-treated animals was significantly less than that of the controls and only slightly less than that of the animals treated with the suspending agent. The average weight of regional lymph nodes in the area adjoining the BCG cold abscess, and of the spleens, livers, and the lungs in the hormone-treated animals showed no significant deviations from that of the controls (table 4). The macroscopic as well as the microscopic picture of these organs failed to show signs of enhanced pathogenicity of BCG due to the prolonged cortisone treatment. The adrenal glands, however, were significantly lighter in the hormone-treated animals than in the controls, and on section they showed uniform atrophy with thinning of the *zona fasciculata*. Cultures of lymph

TABLE 4

EFFECT OF CORTISONE ON WEIGHT OF LYMPH NODES, ADRENALS, SPLEEN, LIVER, AND LUNGS THREE MONTHS AFTER SENSITIZATION WITH BCG VACCINE

(Twelve Animals in Each Group)

| MATERIAL | LIVING BCG VACCINE | | | KILLED BCG VACCINE | | |
|---|---|---|---|---|---|---|
| | Cortisone | Suspensoid | Untreated | Cortisone | Suspensoid | Untreated |
| | Average Weight (Gm.) | | | | | |
| Animal's body weight | 621 | 653 | 690* | 615 | 658 | 699* |
| BCG primary abscess | 4.94 | 3.68 | 4.60 | 3.56 | 2.72 | 3.73 |
| Left superior inguinal nodes | 0.19 | 0.24 | 0.20 | 0.16 | 0.18 | 0.19 |
| Left deep inguinal nodes | 0.08 | 0.10 | 0.10 | 0.07 | 0.09 | 0.10 |
| Left para-iliac nodes | 0.13 | 0.18 | 0.19 | 0.13 | 0.17 | 0.18 |
| Tracheobronchial nodes | 0.29 | 0.30 | 0.30 | 0.28 | 0.27 | 0.29 |
| Total lymph nodes | 1.16 | 1.28 | 1.22 | 1.14 | 1.19 | 1.24 |
| Adrenals | 0.25 | 0.36* | 0.38* | 0.24 | 0.34* | 0.33* |
| Spleen | 0.78 | 0.74 | 0.80 | 0.70 | 0.73 | 0.78 |
| Liver | 39.28 | 30.82 | 34.45 | 36.87 | 30.66 | 34.06 |
| Lungs | 5.40 | 4.86 | 5.26 | 4.73 | 4.89 | 4.75 |

* Significantly different from the cortisone group at $P \leqq 0.01$ level.

nodes and visceral organs showed no greater diffusion of BCG organisms in the hormone-treated than in the untreated controls.

*Immunogenic response to a challenge infection:* The degree of specific resistance against a challenge infection was determined in the surviving animals, which included 24 immunized with living BCG, half of which were cortisone treated, and 24 with killed BCG, half of which were cortisone treated. Twelve normal controls, each weighing 643 gm. plus-minus 56.12 gm., were included. The challenge inoculation consisted of 0.005 mg. of H37Rv (of established virulence) injected intramuscularly in the right thigh. This dose yielded 5,325 living elements (colonies) on Löwenstein egg medium.

Death from generalized and advanced tuberculosis occurred in 8 control animals (67 per cent) between 85 and 117 days, in 2 animals (17 per cent) in both groups treated with cortisone between 109 and 117 days, in 3 animals (25 per cent) in the group immunized with living BCG between 108 and 121 days, and in 5 animals (42 per cent) in the group immunized with killed BCG between 87 and 116 days. The survival data indicate that the lowest degree of resistance was present in the animals inoculated with killed BCG, and that cortisone treatment enhances immunization with both living and killed BCG. The surviving animals were killed between 125 and 130 days after the challenge inoculation.

The necropsy findings are summarized in table 5. The gross pathologic picture showed the fewest tuberculous lesions in the group treated with living BCG and cortisone (67 per cent showed no gross tuberculosis), and somewhat more in the group treated with living BCG alone (50 per cent showed no gross tuberculosis). It is also interesting to observe that the average body weights were highest in these two groups. The quantitative data on regional lymph nodes and visceral

TABLE 5

POST-CHALLENGE FINDINGS OF TUBERCULOUS HYPERTROPHY IN GUINEA PIGS IMMUNIZED WITH BCG AND TREATED WITH CORTISONE DURING THREE POST-VACCINATION MONTHS

(Twelve Animals in Each Group)

| MATERIAL | CONTROLS | LIVING BCG VACCINE | | KILLED BCG VACCINE | |
|---|---|---|---|---|---|
| | | Cortisone | No Treatment | Cortisone | No Treatment |
| | Average Weight (Gm.) | | | | |
| Animal's body weight | 653 | 906*† | 864* | 798* | 786* |
| No gross tuberculous lesions (number) | 0 | 8 | 6 | 1 | 0 |
| Localized tuberculous lesions (number) | 4 | 3 | 3 | 6 | 7 |
| Generalized tuberculous lesions (number) | 8 | 1 | 3 | 5 | 5 |
| Right superior inguinal nodes | 0.76 | 0.12*† | 0.21* | 0.43* | 0.59 |
| Right deep inguinal nodes | 0.29 | 0.08* | 0.09* | 0.37 | 0.34 |
| Right para-iliac nodes | 1.17 | 0.39*† | 0.53* | 0.84 | 0.93 |
| Total lymph nodes | 8.19 | 2.04*† | 2.98* | 6.57 | 7.03 |
| Spleen | 4.07 | 0.90*† | 1.26* | 2.63* | 2.85 |
| Liver | 46.18 | 38.53* | 41.64 | 42.84 | 43.18 |
| Lungs | 9.41 | 6.83* | 7.93* | 9.06 | 8.86 |

* Significantly different from the control group at $P \leq 0.01$ level.

† Significantly different from the living BCG-no treatment group at $P \leq 0.01$ level.

organs support the macroscopic findings. Most of the regional lymph nodes as well as the spleens in the group treated with living BCG and cortisone weighed significantly less than those in the animals immunized with living BCG but untreated. The immunogenic response produced by the killed BCG with or without cortisone treatment was inferior to that observed in the groups immunized with living BCG. The over-all impression is that prolonged cortisone treatment during the pre-challenge period enhances significantly the immunogenic activity of living BCG and to a lesser degree enhances the activity of killed vaccine.

## SUMMARY

Administration of cortisone to guinea pigs inoculated with living or killed BCG vaccine produced a significant reduction in the inflammatory response to BCG and to tuberculin and restricted the intradermal spread of a vital dye.

The hemogram of BCG-sensitized animals treated with cortisone showed early eosinopenia, accelerated lymphopenia, and monocytosis (increased monocyte/lymphocyte ratio), but no sustained changes in the total erythrocytes and leukocytes, hemoglobin, or erythrocyte sedimentation rate.

Necropsy material from BCG-sensitized animals treated with cortisone showed a significant reduction of hyperplasia in regional and distal lymph nodes and in the spleen, marked adrenal atrophy, but no sign of exacerbation of BCG lesions.

Cortisone treatment during the active period of sensitization with BCG produced a significant increase in specific resistance against a challenge infection, which was most marked in animals sensitized with living BCG.

No other evidence was found that the administration of cortisone during and after the active phase of sensitization augmented the virulence of BCG.


## SUMARIO

*El Efecto de la Cortisona sobre la Infección BCG en el Cobayo*

La administración de cortisona a los cobayos inoculados con vacuna BCG viva o muerta produjo una reducción importante en la respuesta inflamatoria a BCG y a la tuberculina, y limitó la difusión intradérmica de un colorante vital.

El hemograma de los animales BCG-sensibilizados y tratados con cortisona reveló eosinopenia temprana, linfopenia acelerada y monocitosis (aumento de la proporción entre monocitos-linfocitos), pero no alteraciones sostenidas en el total de eritrocitos y leucocitos, hemoglobina o coeficiente de eritrosedimentación.

El material autópsico procedente de los animales BCG-sensibilizados y tratados con cortisona mostró una importante reducción de la hiperplasia en los ganglios linfáticos regionales y distales y en el bazo, y notable atrofia suprarrenal, pero sin signos de exacerbación de las lesiones del BCG.

La cortisonoterapia durante el período activo de sensibilización con BCG produjo un aumento importante de la resistencia específica a una infección provocativa, el cual fué más acentuado en los animales sensibilizados con BCG vivo.

No se descubrieron, pues, pruebas de que la administración de cortisona durante la fase activa de la sensibilización y después realce la virulencia del BCG.

## RESUME

*L'action de la cortisone sur l'infection provoquée par le BCG chez le cobaye*

Chez le cobaye inoculé avec le vaccin BCG, préparé avec des bacilles vivants ou tués, l'administration de cortisone produit une réduction significative de la réaction inflammatoire au BCG et à la tuberculine et a limité la diffusion intradermique d'un colorant tissulaire.

Dans l'hémogramme d'animaux sensibilisés au BCG et traités par la cortisone on observe une éosinopénie précoce, une lymphopénie accélérée et une monocytose (accroissement du rapport monocyte-lymphocyte), mais aucune modification constante du nombre total des érythrocytes, des leucocytes, du taux d'hémoglobine ou de la vitesse de sédimentation globulaire.

Dans les pièces d'autopsie prélevées chez des animaux sensibilisés au BCG et traités par la cortisone on observait une nette réduction de l'hyperplasie des ganglions lymphatiques régionaux ou distants et de la rate; une atrophie marquée des surrénales, mais nul signe d'un accroissement des lésions imputables au BCG.

Le traitement par la cortisone durant la période active de la sensibilisation par le BCG a produit une augmentation significative de la résistance spécifique à l'infection provoquée; c'est chez les animaux sensibilisés avec BCG préparé avec des bacilles vivants que la résistance était la plus marquée.

Aucune preuve démontrant que l'administration de la cortisone, pendant et après la phase active de sensibilisation, augmente la virulence du BCG n'a été obtenue.


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# VIOMYCIN THERAPY IN HUMAN TUBERCULOSIS[1, 2]

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## INTRODUCTION

Viomycin is an antimicrobial produced by an actinomycete named, variously, *Streptomyces puniceus* and *Streptomyces floridae* by the two groups of investigators who independently discovered and developed the drug (1, 2). A survey of its antibacterial properties indicates that it is active chiefly against mycobacteria, including the tubercle bacillus, which it suppresses both *in vitro* and *in vivo*. Viomycin is as active against streptomycin-resistant as it is against streptomycin-susceptible tubercle bacilli, both in the test tube and in animals (3, 4, 5, 6). It has been found possible easily to develop resistant strains by serial passage of tubercle bacilli through medium containing increasing concentrations of viomycin (6). The isolation of resistant strains of tubercle bacilli from experimental animals after sixty to seventy days of treatment with viomycin has been reported (6). Studies of the effect of viomycin on tuberculosis in experimental animals demonstrated marked suppression of the disease comparable with the effect of streptomycin, although higher dosage with the former is required (6, 7). The toxicity in animals was sufficiently low to warrant a trial in humans (8).

Investigation in human subjects revealed that, with daily doses between 30 and 75 mg. per kg. of body weight, given for as long as 182 days, certain toxic manifestations could be expected. These were renal irritation (albuminuria, cylindruria, mild azotemia), abnormalities of plasma electrolytes (chiefly hypocalcemia and hypokalemia), impairment of vestibular function, occasional instances of partial deafness, and minor hypersensitivity reactions (9).

## METHODS

The present report deals with the therapeutic results and experience with 23 cases of human tuberculosis treated with viomycin sulfate.[3] In the beginning of the study in August, 1950, the first patients chosen were selected because they were known to have tubercle bacilli resistant to streptomycin or had done poorly on original treatment with streptomycin and para-aminosalicylic acid (PAS). As experience developed and it was perceived that viomycin had some therapeutic value, patients with more acute disease who had not had previous antimicrobial therapy were included.

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[2] This study was aided, in part, by a grant from Chas. Pfizer & Co., Inc., Brooklyn, New York.

[3] Viomycin sulfate was supplied by Chas. Pfizer & Co., Inc., Brooklyn, New York.

*Dosage regimen:* In the first 16 cases, the drug was given in a daily dosage of 50 to 60 mg. per kg. of body weight for periods of 30 to 126 days. In *Case 17*, the drug was given daily for 101 days, then daily or twice weekly for 162 days. In the last 6 cases the drug was administered in dosage of 50 to 60 mg. per kg. of body weight daily for 30 days, then twice weekly for total periods varying from 69 to 144 days. In one patient (*Case 23*), PAS was given daily with viomycin. The daily viomycin dose varied from 2.0 to 4.0 gm. and was divided equally into two separate intramuscular (gluteal) injections given at 9:00 a.m. and at 4:00 p.m.

*Type of tuberculosis:* Twenty-one of the 23 patients had far advanced pulmonary tuberculosis. Extrapulmonary tuberculosis was present in 9 of the 23 patients and included one case of tuberculous laryngitis, one of severe ulcerative tuberculous endobronchitis, one of intestinal tuberculosis, one of tuberculous fecal fistulas, one of post-laryngectomy tuberculous cellulitis of the neck, one of tuberculous cervical lymphadenitis, and 3 cases of draining tuberculous cutaneous sinuses.

*Previous chemotherapy:* Of the 23 patients, 8 had never received streptomycin or para-aminosalicylic acid prior to viomycin therapy. Fifteen had received streptomycin alone or in conjunction with PAS: 11 of these had streptomycin-resistant organisms at the start of viomycin treatment, 8 and 3 being resistant, respectively, to 200 $\gamma$ per ml. and 15 $\gamma$ per ml.

*Clinical laboratory observations:* In each case, prior to viomycin therapy, a history was obtained and a complete physical examination was performed, including indirect laryngoscopy and neurologic examination. Conventional chest roentgenograms were obtained with lateral and special views, as indicated. The sputum was examined microscopically in smears, and cultures of concentrates were made. A complete blood cell count, urinalysis, erythrocyte sedimentation rate (Westergren method), measurement of blood urea nitrogen, and electrocardiogram were obtained. Plasma sodium and potassium concentrations were determined with a flame photometer. These clinical, roentgenographic, and laboratory examinations were repeated at intervals varying from one to four weeks during treatment. When indicated, serum albumin and globulin concentrations were determined, and liver function and other tests were made to help guide clinical management. Auditory and vestibular functions were measured by audiometric and caloric tests at the beginning and end of therapy.

In the first 6 cases of the series, plasma sodium, plasma potassium, blood calcium, plasma chloride, plasma bicarbonate, and serum phosphorus concentrations were determined weekly during therapy. These observations did not differ materially from those reported earlier by Werner and associates (9). Only the data on potassium concentrations will be presented.

*General management:* All patients were kept at bed rest during viomycin treatment, and no collapse of the lung or other major therapeutic procedures were employed during the study period. In view of an earlier report of plasma electrolyte disturbances (9), some of the patients were given oral maintenance doses of potassium chloride, 1.0 gm. three times daily, and calcium lactate, 0.6 gm. three times daily, over variable and irregular periods. Temperatures were measured rectally three times daily. Patients were weighed at the beginning of, and weekly during, treatment.

## OBSERVATIONS

### *Therapeutic Results*

The race, sex, and age of each patient in the series, together with description of the pathology and extent of the tuberculous lesions, are listed in table 1. Pertinent data concerning previous treatment are recorded. The dosage regimen and duration of viomycin treatment, together with the clinical, roentgenographic, and bacteriologic response to therapy, are likewise recorded. Of the 23 cases in the series so recorded, detailed descriptions of 5 (*Cases 2, 10, 13, 9,* and *17*), together with roentgenographic and photographic illustrations, are presented in the case reports below. These 5 cases were selected for detailed presentation, not because of the therapeutic results obtained, but rather because they are exemplary of the entire group reported in table 1.

In table 2 the evaluations of the therapeutic effects of viomycin on various sites of tuberculosis are recorded. As several of the patients in the series had more than one site of tuberculous involvement, the number of sites is greater than the number of patients. In forming an evaluation of the effect of viomycin on each site, the data were reviewed by several examiners and the final opinion was recorded, using the terms *marked improvement, moderate improvement, slight improvement,* and *unchanged.* In no instance was it believed that a lesion became worse as a result of viomycin therapy, although some lesions became worse by reason of the natural course of the disease.

Similarly, in table 3, the evaluations of the effect of viomycin on the over-all course of the tuberculous disease are recorded. The same terminology is used.

### *Drug Toxicity*

Table 4 is a composite summary of all toxic manifestations noted in this series. Deafness was the most serious. As noted in the table, audiometric measurements were suitable for evaluation in only 14 patients; of these, 11 showed definite high frequency hearing loss characteristic of nerve deafness. In spite of the high incidence of nerve deafness by audiometry, actual conversational hearing loss was noted in only 2 patients. Their deafness was of moderate degree and did not appear to progress or regress after the cessation of therapy. No cases of total deafness were observed.

The next most serious toxic manifestation was hypokalemia. Figure 1 is a scatter-graph showing all of the available serial plasma potassium levels with relation to duration of therapy. It is seen that slight depression of plasma potassium was not unusual and that the greatest reduction frequently occurred after the cessation of therapy. The present studies were not sufficient to reveal the mechanism of the hypokalemia. The most severe reduction occurred in a patient who, in addition to pulmonary tuberculosis, also had intestinal tuberculosis and amyloid nephrosis. This patient and one other had extreme weakness, heart sounds of poor quality, and electrocardiographic changes consistent with hypokalemia during the period of potassium depression; they required parenteral potassium therapy intermittently during a period of two weeks.

No serious alteration in other plasma electrolytes was observed in the series. There was no difference with regard to electrolyte disturbances between those patients who received and those who did not receive oral potassium and calcium supplements. There were no instances of hypocalcemic tetany.

Other minor reactions to viomycin therapy are listed in table 4.

### *Bacterial Resistance*

It was not possible to obtain serial studies of bacterial drug susceptibility. The few studies which it was possible to make merely indicate that drug-resistant tubercle bacilli do emerge during viomycin therapy. The data were not sufficient to determine the frequency, speed of emergence, or degree of resistance.

### *Case Reports*

*Case 2* (M. W.). A 31-year-old, married, Negro male shipping clerk was admitted to Bellevue Hospital on October 1, 1949, with symptoms of eleven months' duration, consisting of productive cough, malaise, night sweats, fever, and weight loss of 36 pounds.

*Physical examination:* The patient's rectal temperature was 102°F.; pulse rate, 96 per minute; respiratory rate, 30 per minute; and blood pressure, 100 systolic and 60 diastolic in mm. of mercury. The patient was well developed but extremely emaciated, appearing chronically ill, with frequent cough productive of thick yellow sputum. The significant findings were in the chest. The percussion note was dull over the entire left lung. Anteriorly, amphoric breath sounds were audible beneath the left clavicle. Bronchophony with whispered pectoriloquy was noted in the same area. There were numerous moist coarse rales over the left lung, with numerous consonating rales in the left infraclavicular region. The right lung was clear on percussion and auscultation.

The chest roentgenogram showed elevation of the left diaphragm, with some mediastinal shift to the left. A homogeneous confluent infiltrate was observed throughout the left lung except for an 8 cm. cavity occupying the upper lobe. There were diffuse lobular pneumonic infiltrates in the upper two-thirds of the right lung.

*Laboratory findings:* The sputum revealed tubercle bacilli on microscopic examination and culture. The organisms isolated initially were susceptible to streptomycin and PAS. The other laboratory tests yielded values within normal limits.

*Course:* The patient received 91 days of treatment with streptomycin chemically combined with PAS, 1.8 gm., daily, from November 26, 1949, to January 24, 1950. There was marked symptomatic improvement but essentially no roentgenographic change. By the fourteenth week, tubercle bacilli cultured from the patient's sputum were resistant to streptomycin in a concentration of 100 $\gamma$ per ml. but remained susceptible to PAS, 0.1 $\gamma$ per ml. In the post-treatment period, the patient remained afebrile and held a stationary weight; the sputum averaged 33 cc. daily. He was re-treated with streptomycin, 1.0 gm., and PAS, 12 gm., daily for 84 days, from May 19 to August 11, 1950. There was no change in the clinical course except for a weight loss of 7 pounds. The roentgenograms were essentially unchanged.

Because of the emergence of streptomycin-resistant tubercle bacilli, the patient was started on viomycin therapy and received 3 gm. daily for 126 days, from August 16 to December 17, 1950. The clinical response was slight. Low fever persisted for six weeks. Thereafter, the patient remained afebrile. There was a temporary weight gain of 4 pounds up to the seventh week, after which there was gradual loss of 11 pounds by the end of treatment. The volume of sputum decreased in the first month of therapy but gradually doubled after the fifteenth week. Tubercle bacilli persisted in smears and cultures. Serial roentgenograms remained essentially unchanged (figures 2*A* and *B*), showing almost complete destruction of the left lung and scattered lobular pneumonic lesions in the upper two-thirds

TABLE 1

TABULAR SUMMARY OF CASE MATERIAL AND THERAPEUTIC RESULTS

| CASE NUMBER, SEX, RACE, AGE | DESCRIPTION OF TUBERCULOSIS | | PREVIOUS ANTIBIOTIC THERAPY | VIOMYCIN | | CLINICAL RESPONSE | ROENTGENOGRAPHIC RESPONSE | BACTERIAL RESPONSE (SPUTUM CONCENTRATES UNLESS OTHERWISE SPECIFIED) | | | OVER-ALL IMPROVEMENT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pulmonary | Extrapulmonary | | Dose per Day | Total Days | | | | Before | After | | |
| 1. F, N, 19 | Caseous and exudative lesions entire right lung with cavitation RUL. | None | SM 1 gm. / PAS 12 gm. 43 days ended 9 days before VM therapy. | 3 gm. | 126 | Became healthy in appearance; afebrile after 7 weeks; gained 12 pounds; sputum volume decreased from 60 cc. per day to none. | Marked clearing with residual RUL cavity. | Smear<br>Culture | +<br>+ | 0<br>+ | Marked | Came to uneventful right thoracoplasty 6 months later on SM and PAS. |
| 2.* M, N, 32 | Caseous and cavitary disease, entire left lung, giant cavity in LUL, scattered lobular pneumonic infiltrates in right lung. | None | SM 1 gm., 91 days; SM 1 gm. / PAS 12 gm. 84 days ended 14 days before VM therapy. | 3 gm. | 126 | Remained chronically ill; afebrile after 6 weeks, but fever recurred in sixteenth week; lost 9 pounds; sputum increased from 25 to 60 cc. daily. | Unchanged | Smear<br>Culture | +<br>+ | +<br>+ | None | |
| 3. M, N, 52 | Contracted RUL containing multiple cavities, scattered caseous infiltrates with small cavities throughout both lungs. | None | SM 1 gm., 91 days; SM 1 gm. / PAS 12 gm. 43 days ended 14 days before VM therapy. | 3 gm. | 126 | Some improvement in appearance; afebrile after 3 weeks; gained 4 pounds; sputum volume reduced. | Further contraction of RUL; slight clearing of lower right lung field. | Smear<br>Culture | +<br>+ | +<br>+ | Slight | |
| 4. M, N, 49 | Mixed exudative and caseous disease throughout right lung with cavitation RUL; scattered exudative lesions throughout left lung. | None | SM 1 gm. / PAS 12 gm. 97 days ended 14 days before VM therapy. | 4 gm. | 95 | Became healthy in appearance; afebrile after 5 weeks; gained 35 pounds; sputum volume decreased. | Marked bilateral clearing; residual cavitation RUL. | Smear<br>Culture | 0<br>0 | 0<br>+ | Marked | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5. M, W, 39 | Bilateral caseous and cavitary disease chiefly in RUL and LUL. | None | SM 1 gm. } 91 days<br>PAS 9 gm. }<br>SM 1 gm. } 34 days<br>PAS 12 gm. }<br>ended 14 days before VM therapy. | 4 gm. | 80 | Remained chronically ill; afebrile after 4 weeks; lost 8 pounds; sputum volume decreased. | Right lung unchanged; left lung cavities increased in size. | Smear<br>Culture | +<br>+ | +<br>+ | None | |
| 6. F, N, 31 | Exudative and caseous disease RUL with 3.5 cm. cavity. | None | None | 2 gm. | 36 | Improved general appearance; afebrile after first week; gained 2 pounds; sputum disappeared. | Cavity smaller; slight clearing. | Smear<br>Culture | 0<br>+ | 0<br>+ | Slight | Left hospital against advice. |
| 7. M, W, 42 | Exudative disease RUL and RLL. (Onset with hemoptysis) | None | None | 2 gm. | 92 | Marked improvement in appearance; afebrile after 2 weeks; gained 15 pounds; sputum disappeared. | Moderate clearing. | Smear<br>Culture | +<br>+ | 0<br>+ | Moderate | |
| 8. M, W, 50 | Caseous and cavitary disease entire right lung and LUL. | Ileocecal tuberculosis | None | 3 gm. | 30 | Remained chronically ill; remained febrile. Sputum volume markedly decreased. Intestinal symptoms subsided rapidly and completely. | Slight clearing. | Smear<br>Culture | +<br>+ | +<br>+ | Slight | Had amyloidosis with nephrotic syndrome as a complication. |
| 9.* M N, 39 | None | Subsiding right pleurisy with effusion; tuberculosis of left ninth and tenth ribs with draining cutaneous sinus for 2 years; Pott's abscess with surgical fusion of vertebrae D8 to D12. | SM, 1 gm., 156 days, ended 2 years before VM therapy. | 3 gm. | 91 | Became healthy in appearance. Gained 11 pounds; draining sinus closed within 3 months. | No change in appearance of ribs. | Smear (sinus)<br>Culture (sinus) | 0<br>+ | 0<br>0 | Marked | Left hospital against advice. |

TABLE 1—*Continued*

| CASE NUMBER, SEX, RACE, AGE | DESCRIPTION OF TUBERCULOSIS | | PREVIOUS ANTIBIOTIC THERAPY | VIOMYCIN | | CLINICAL RESPONSE | ROENTGENOGRAPHIC RESPONSE | BACTERIAL RESPONSE (SPUTUM CONCENTRATES UNLESS OTHERWISE SPECIFIED) | | | OVER-ALL IMPROVEMENT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pulmonary | Extrapulmonary | | Dose per Day | Total Days | | | | Before | After | | |
| 10.* M, N, 36 | Exudative and caseous disease with cavitation LUL; small exudative infiltrates in RUL. (Onset with hemoptysis) | None | None | 4 gm. | 71 | Became healthy in appearance; afebrile after third week; gained 6 pounds. Marked reduction in sputum volume. | Moderate clearing with residual LUL cavitation. | Smear<br>Culture | +<br>+ | +<br>+ | Marked | |
| 11. M, W, 52 | Bilateral caseous cavitary disease, maximal LUL, throughout both lungs. | Tuberculosis, right epididymis with scrotal sinus draining for 2 months. Diffuse genitourinary tuberculosis | SM, 1 gm., 38 days, ended 3½ years before VM therapy. | 3 gm. | 48 | Appearance improved but still chronically ill; fever reduced; gained 8 pounds; scrotal sinus closed by end of therapy. | Unchanged | Smear<br>Culture<br>Smear (sinus)<br>Culture (sinus) | +<br>+<br>0<br>+ | +<br>+<br>0<br>0 | Moderate | Because of marked involvement of the right epididymis and testicle, a right orchiectomy was done 6 weeks after end of VM therapy; on SM and PAS. |
| 12. M, W, 60 | Caseous and cavitary disease with giant cavity LUL with small caseous infiltrate in RUL. | None | SM 1 gm. } 90<br>Promisole } days<br>SM 1 gm. } 42<br>PAS 12 gm. } days<br>SM 1 gm., 42 days, ended 1 week before VM therapy | 2 gm. | 92 | Remained chronically ill; remained febrile; sputum volume decreased but remained high. | Increased cavitation LUL; right unchanged. | Smear<br>Culture | +<br>+ | +<br>+ | None | |
| 13. F, N, 34 | Mixed exudative and caseous disease entire right lung with RUL cavitation. | None | None | 3 gm. | 93 | Became healthy in appearance; afebrile after 4 weeks; gained 10 pounds. Sputum volume markedly reduced. | Marked clearing, diffuse residual caseous foci, RUL cavitation persisted. | Smear<br>Culture | +<br>+ | +<br>+ | Marked | Came to uneventful right pneumonectomy 5 months after end of VM therapy; on SM and PAS. |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14. F, W, 21 | Bilateral upper lobe caseous cavitary disease with small exudative lesions in upper two-thirds of both lungs. | Tuberculosis of cervical lymph nodes, bilateral. | None | 2 gm. | 93 | Became healthy in appearance; afebrile after 2 weeks; gained 32 pounds; cervical nodes decreased in size. | Moderate clearing of exudative lesions, marked decrease in size of cavities. | Smear<br>Culture | +<br>+ | 0<br>+ | Moderate | Later treated with SM and PAS and came to bilateral resection of cervical nodes; resection of RUL and RML and segmental resection LUL performed uneventfully 15 months later. |
| 15. M, W, 28 | Exudative disease RUL with caseous focus RUL. (Onset with hemoptysis) | None | None | 3 gm. | 92 | Remained healthy in appearance; afebrile after 2 weeks; gained 11 pounds; sputum disappeared. | Complete clearing of exudative disease; small residual cavity RUL. | Smear<br>Culture | 0<br>+ | 0<br>0 | Marked | |
| 16. F, W, 36 | Diffuse, fine, discrete exudative disease throughout both lungs, small cavities both upper lobes. | Tuberculous laryngitis | SM 1 gm. / Promizole } 120 days<br>SM 1 gm. / PAS 12 gm. } 66 days<br>ended 1 week before VM therapy. | 2 gm. | 77 | Laryngeal symptoms subsided within 2 weeks, and larynx became normal; remained healthy in appearance; sputum disappeared. | Moderate clearing, cavities decreased in size. | Smear<br>Culture | +<br>+ | 0<br>0 | Moderate | Later roentgenograms cleared almost completely after prolonged PAS. |
| 17.* F, W, 36 | Caseocavitary disease LUL; less extensive caseocavitary disease RUL. | Tuberculous cellulitis of neck with pharyngeal fistula (complication of laryngectomy for squamous cell carcinoma of larynx). | SM 1 gm. / PAS 9 gm. } 91 days<br>SM 1 gm. / PAS 12 gm. } 90 days<br>ended two months before VM therapy. | 3 gm. followed by 3 gm. O.D. or b.i.w. | 101<br>162 | Became healthy in appearance; afebrile after 4 weeks; gained 13 pounds; neck wound healed after 2 months of treatment. | Moderate bilateral clearing; decrease in size of cavities. | Smear<br>Culture | +<br>+ | 0<br>0 | Marked | Uneventful thoracoplasty started at the end of VM treatment. |

TABLE 1—*Concluded*

| CASE NUMBER, SEX, RACE, AGE | DESCRIPTION OF TUBERCULOSIS | | PREVIOUS ANTIBIOTIC THERAPY | VIOMYCIN | | CLINICAL RESPONSE | ROENTGENOGRAPHIC RESPONSE | BACTERIAL RESPONSE (SPUTUM CONCENTRATES UNLESS OTHERWISE SPECIFIED) | | | OVER-ALL IMPROVEMENT | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pulmonary | Extrapulmonary | | Dose per day | Total days | | | | Before | After | | |
| 18. F, N, 19 | Cavernous cavitary disease entire left lung, exudative infiltrates in right lung. | Endobronchial tuberculosis (left main stem bronchus with severe ulceration and granulation). | SM 1 gm., 42 days<br>PAS 12 gm., 90 days<br>SM 1 gm. } 330<br>PAS 12 gm. } days | 2 gm.<br>2 gm.<br>b.i.w. | 30<br>50 | Healing of endobronchial disease after 4 weeks; cicatrizing bronchostenosis after 11 weeks; from critical condition became healthy in appearance; high fever reduced to low; sputum decreased; but effects not sustained. | Progressive cavitation on left, diffuse spread to right lung after third bronchoscopy. | Smear<br>Culture | +<br>+ | +<br>+ | None | Uneventful left pneumonectomy on INH 10 weeks after end of VM treatment. |
| 19. F, N, 19 | Post-thoracoplasty exudative spread entire right lung; bilateral upper lobe cavernous cavitary disease. | None | SM 1 gm. } 720<br>PAS 12 gm. } days | 2 gm.<br>3 gm.<br>b.i.w. | 30<br>30 | From critical condition course improved; high fever reduced to low; gained 12 pounds; sputum volume reduced. | Upper lobe lesions unchanged; very little clearing; caseous residuum RLL. | Smear<br>Culture | +<br>+ | +<br>+ | Slight | No improvement on subsequent INH; left hospital against advice. |
| 3 . F, N, 32 | Bronchopleural cutaneous fistula (derived from Pott's abscess of spine); fistula closed at time of VM treatment but sinus remained. | Pott's abscess lower dorsal spine; tuberculosis of ribs; draining tuberculous sinuses, right posterior chest (old decortication of pleura and costectomy). | SM 1 gm., 21 days<br>SM 1 gm. } 126<br>PAS 12 gm. } days<br>PAS 12 gm., 84 days | 2 gm.<br>2 gm.<br>b.i.w. | 30<br>94 | Drainage gradually decreased; sinuses closed in fourth month; improved sense of well-being; gained 9 pounds. | | Smear (sinus)<br>Culture (sinus) | 0<br>+ | 0<br>0 | Marked | Sinus reopened slightly one month after end of treatment but closed again after 2 weeks of retreatment with VM. |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21. F, N, 45 | Diffuse, fine, exudative and caseous disease upper half both lung fields; multiple cavities left mid-lung. | Tuberculous endometritis; ileocecal tuberculosis; fecal fistulae (2). | SM 1 gm. / PAS 12 gm. } 30 days | 2 gm.<br>2 gm.<br>b.i.w. | 30<br>144 | Dramatic improvement in general appearance; gained 16 pounds; marked decrease in drainage and size of fistulae but closure incomplete. | Cavities decreased in size; slight bilateral clearing. | Culture (gastric) | + | + | Moderate | |
| 22. M, Y, 48 | Caseocavitary disease LUL with exudative lesions left base; small caseous infiltrate RUL. | None | None | 2 gm.<br>2 gm.<br>b.i.w. | 30<br>51 | Lost toxic appearance; high fever reduced to low; gained 9 pounds; sputum decreased. | Slight progression of disease in left lung with further caseation and slight increase in size of cavities; right unchanged. | Smear<br>Culture | +<br>+ | +<br>+ | None | Failed to improve by roentgenogram on subsequent combined antimicrobial therapy (SM, INH). |
| 23. M, N, 37 | Caseocavitary disease LUL, giant cavity LUL; diffuse, fine, exudative and caseous lesions left lower and right mid-lung field | None | SM 1 gm. / PAS 12 gm. } 253 days<br>Isoniazid 300 gm., 56 days | 4 gm. (with PAS 12 gm./day)<br>4 gm. b.i.w. (with PAS 12 gm./day) | 30<br>30 | Remained chronically ill and febrile; lost 13 pounds. | Giant cavity larger; slight clearing in right mid-lung. | Smear<br>Culture | +<br>+ | +<br>+ | None | |

* Detailed case reports presented.
\+ positive; 0 negative

RUL—right upper lobe
RML—right middle lobe
RLL—right lower lobe
LUL—left upper lobe
LLL—left lower lobe
SM—streptomycin
PAS—para-aminosalicylic acid
VM—viomycin
INH—isoniazid
O.D.—once daily
b.i.w.—twice weekly

TABLE 2

EVALUATION OF EFFECT OF VIOMYCIN ON 30 SITES OF TUBERCULOSIS IN 23 PATIENTS

| | |
|---|---|
| Pulmonary tuberculosis (roentgenographic evaluation): | |
| Marked improvement | 4 |
| Moderate improvement | 5 |
| Slight improvement | 4 |
| Unchanged | 8 |
| Laryngeal tuberculosis: | |
| Marked improvement | 1 |
| Endobronchial tuberculosis: | |
| Moderate improvement | 1 |
| Intestinal tuberculosis: | |
| Moderate improvement | 1 |
| Tuberculous fecal fistulas: | |
| Moderate improvement | 1 |
| Tuberculous cellulitis of neck (post-laryngectomy for carcinoma): | |
| Marked improvement | 1 |
| Lymph node tuberculosis: | |
| Moderate improvement | 1 |
| Draining tuberculous sinuses: | |
| Marked improvement | 3 |

TABLE 3

OVER-ALL EVALUATION OF EFFECT OF VIOMYCIN ON COURSE OF TUBERCULOUS DISEASE IN 23 PATIENTS

| | NUMBER OF PATIENTS |
|---|---|
| Marked improvement | 8 |
| Moderate improvement | 5 |
| Slight improvement | 4 |
| Unchanged | 6 |

of the right lung. At the end of therapy the patient's course continued to be that of slow wasting. He was placed on PAS, 12 gm. daily, and transferred to a sanatorium for further care.

This case exemplifies the failure of tuberculostatic drug therapy to change materially the course of pulmonary tuberculosis in which tissue destruction was the prominent feature.

*Case 10* (F. R.). A 36-year-old, married, Negro male, was admitted on March 30, 1951, because of gross hemoptysis for four days. The present illness began approximately three months before admission with a dry cough becoming productive of mucopurulent sputum in the month prior to admission, with an associated weight loss of 5 pounds. The hemoptyses were estimated at approximately three cupfuls daily in the four days preceding admission.

*Physical examination:* The patient's rectal temperature was 104°F.; pulse rate, 120 per minute; respiratory rate, 32 per minute; and blood pressure, 150 systolic and 90 diastolic in mm. of mercury. The patient was well developed and well nourished and rather apprehensive. The important findings were limited to the chest. Dullness to percussion and

TABLE 4

TOXIC REACTIONS EXPERIENCED IN TREATING 23 PATIENTS WITH VIOMYCIN

| REACTION | NUMBER OF PATIENTS |
|---|---|
| Total deafness | 0 |
| Partial conversational hearing loss | 2 |
| High-tone deafness (audiogram) | 11* |
| Dizziness, vertigo, and/or tinnitus | 7 |
| Vestibular loss (caloric test) | 0† |
| Hypokalemia (severe, requiring parenteral restoration) | 2 |
| Hypokalemia (mild, by laboratory measurement only) | 8 |
| Electrocardiographic abnormalities (hypokalemia) | 2 |
| Albuminuria (transient) | 14 |
| Azotemia | 0 |
| Skin eruptions | 0 |
| Eosinophilia | 13 |
| Deaths | 0 |

* Audiogram evaluation unreliable in 9 of the remaining 12 patients because of previous damage or inadequate measurements.

† Vestibular tests unreliable in 10 patients because of previous damage or inadequate measurements, but normal in the remaining 13 patients.

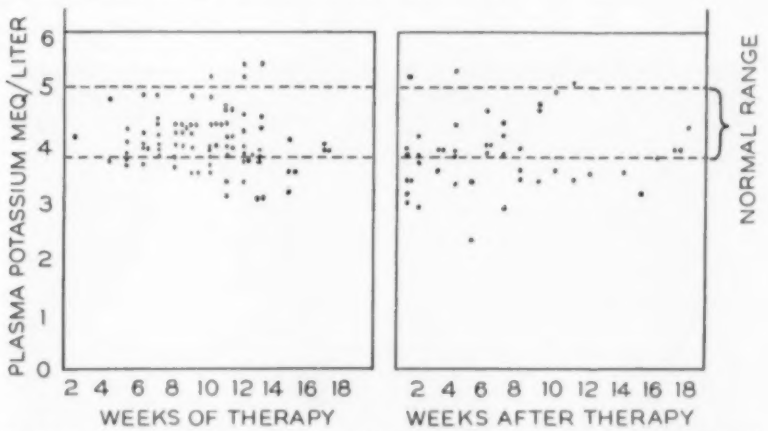


FIG. 1. Scatter-graph of all potassium determinations available during and following viomycin therapy.

bronchovesicular breath sounds were present over the left upper third anteriorly. Numerous fine moist rales were audible throughout the left upper lobe.

The chest roentgenogram was interpreted as showing lobular pneumonic infiltrates in the left upper lobe with several small areas of caseation.

*Laboratory findings:* Smears and cultures of the sputum were positive for tubercle bacilli. The hemoglobin concentration was 11 gm. per 100 ml.; and the leukocyte count was 6,800, with 75 per cent polymorphonuclears.

*Course:* During the first two weeks in which the patient was observed, receiving only bed rest therapy, his temperature remained between 102° and 104°F. During the first four

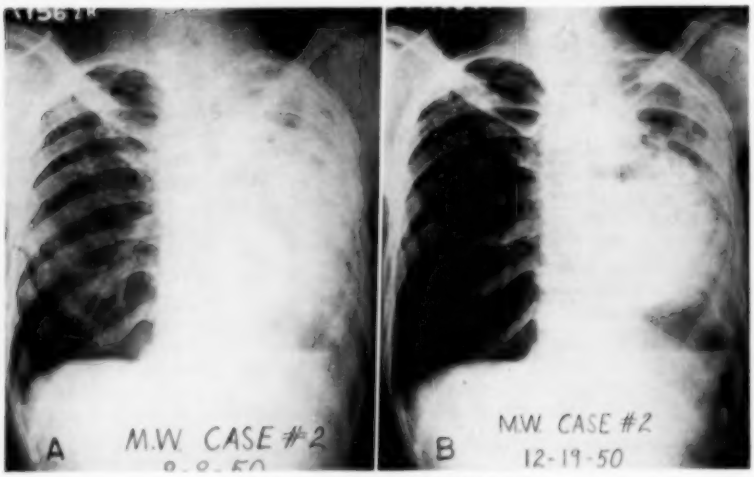


FIG. 2. *Case 2* (M. W.)

*A*. Roentgenogram dated August 8, 1950, prior to institution of viomycin.

*B*. Roentgenogram dated December 19, 1950, immediately after viomycin was discontinued.

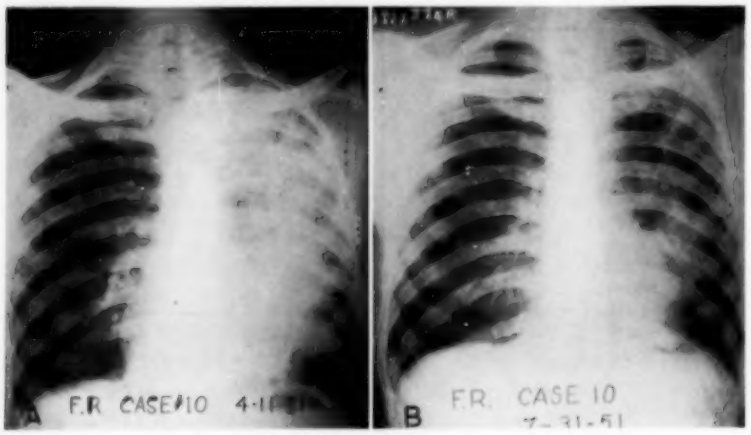


FIG. 3. *Case 10* (F. R.)

*A*. Roentgenogram dated April 11, 1951, prior to institution of viomycin.

*B*. Roentgenogram dated July 31, 1951, six weeks after the end of viomycin therapy.

hospital days he continued to have hemoptyses averaging one to two cupfuls daily. After April 2, 1951, the sputum was non-bloody. Serial chest roentgenograms showed an increase in the extent of tuberculous pneumonia throughout the upper two-thirds of the left lung, with suggestive cavitation at the level of the first and second anterior intercostal spaces and lobular pneumonic infiltrates extending to the base (figure 3*A*). This was believed to

be a fresh spread of disease following hemoptysis. The leukocyte count remained normal. The sputum volume, of mucopurulent character, averaged 45 to 60 cc. daily.

Viomycin therapy was started on April 13, 1951, the fifteenth hospital day, and was continued until June 23, 1951, for a total of 71 days, in dosage of 4 gm. daily. After two weeks of therapy, the patient's temperature had fallen to 100°F. or lower, and he remained essentially afebrile thereafter. The sputum volume gradually decreased from 60 to 15 cc. daily, but the sputum continued to be positive for tubercle bacilli on smear and culture. The patient gained 6.5 pounds and felt stronger. Serial chest roentgenograms showed moderate clearing of the tuberculous pneumonia in the left lung, with persistence of diffuse scattered areas of exudative and caseous disease throughout. Suggestive residual cavitation was noted in the first and second anterior intercostal spaces (figure 3*B*). Viomycin was discontinued because of slight progression of nerve deafness by audiogram. For one and one-half months thereafter the patient remained on bed rest alone, without change in his clinical course or chest roentgenograms. On July 18, 1951, long-term streptomycin and PAS therapy was started, with very slight further clearing roentgenographically after two months of therapy and failure of sputum to become negative for tubercle bacilli by culture.

This case is an example of clearing of a dated posthemoptoic tuberculous pneumonia treated with viomycin. The end result perhaps would have been more striking if viomycin had been instituted earlier.

*Case 13* (M. P.). A 34-year-old Negro housewife was admitted March 7, 1951, complaining of dry cough for four months. Gradually, the cough became productive of half a cupful of mucopurulent sputum daily. Anorexia, with a gradual weight loss of 14 pounds, was noted. Right-sided chest pain, fever, night sweats, and hoarseness were present for six weeks. A roentgenogram of the chest taken two months prior to admission was reported to be normal.

*Physical examination:* The patient's rectal temperature was 103°F.; pulse rate per minute, 120; the respiratory rate was 24 per minute; and blood pressure, 130 systolic and 90 diastolic in mm. of mercury. The patient was a well-developed, poorly nourished Negro woman, acutely and chronically ill, with frequent paroxysms of coughing. Abnormal findings included red, edematous vocal cords on indirect laryngoscopy. Examination of the chest showed dullness over the right upper half with bronchial breath sounds, increased vocal fremitus, consonating rales at the apex, and numerous medium moist rales throughout the right chest, decreasing toward the base. The left lung was clear.

Chest roentgenogram on admission revealed a diffuse confluent infiltrate in the right upper lobe with a large cavity in the posterolateral segment, a smaller cavity anteriorly, and lobular pneumonic infiltrates in the right middle and right lower lobes.

*Laboratory findings:* The sputum was positive for tubercle bacilli on smear and culture. Complete blood cell count and urinalysis were within normal limits. Serologic tests for syphilis: Mazzini 4-plus, Wassermann 2-plus, VDRL positive 1:10. Cerebrospinal fluid examination was normal.

*Course:* The patient's temperature ranged intermittently between 101° and 104.8°F. for seventeen days. She had a severe exhausting cough productive of 120 cc. of sputum daily, with night sweats, anorexia, and further weight loss of 4 pounds. A ten-day course of penicillin, totaling 6,000,000 units, was given for latent syphilis. Since serial roentgenograms revealed increased spread of disease in the right lung (figure 4*A*), viomycin, 2 gm. daily, was started on March 24, 1951. This therapy was continued for 93 days. Within the first week the patient began to feel better, with improvement of appetite. Cough and expectoration decreased. The temperature dropped to the 100°F. range, and night sweats ceased. After one month of therapy, the patient remained afebrile. Cough was infrequent, and the sputum volume was reduced to approximately 15 cc. daily. Hoarseness had disappeared, and the larynx became normal on indirect laryngoscopy. The number of rales heard over

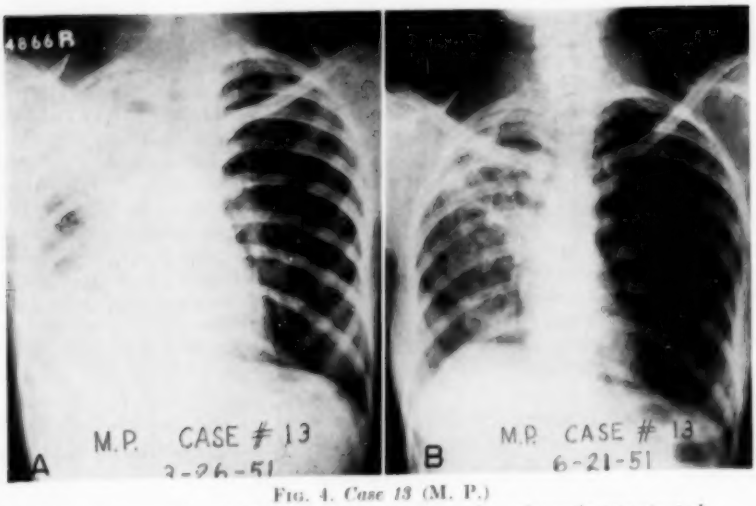


FIG. 4. *Case 13* (M. P.)

*A*. Roentgenogram dated March 26, 1951, two days after viomycin was started.

*B*. Roentgenogram dated June 21, 1951, in the last week of viomycin therapy.

the right lung diminished markedly. The patient gained 10 pounds while under therapy. Her sputum remained positive for tubercle bacilli on culture, but smears became negative. There were no symptoms of drug toxicity, and the only objective finding was slight hearing loss for high frequency tones noted in serial audiograms. Roentgenograms revealed progressive clearing on the right and, by the end of therapy, this was marked. However, scattered caseous residual foci with cavitation persisted in the right upper lobe (figure 4*B*).

When viomycin was discontinued, the patient noticed immediate increase in cough, expectoration, general malaise, and anorexia. A 2-pound weight loss occurred. Her temperature rose to 102° and 104°F. during the next ten days. The roentgenogram revealed enlargement of the cavities in the right upper lobe. Accordingly, streptomycin and PAS were started on July 3, 1951, and continued on a daily regimen until August 13, 1951, when streptomycin administration was changed to twice weekly, but PAS was continued daily. The clinical improvement was maintained. Serial roentgenograms continued to show further clearing on the right, the left lung remaining entirely normal. Five months after the end of viomycin treatment, an uneventful right pneumonectomy was performed. Postoperatively, the patient continued on long-term streptomycin and PAS without complications.

This case represents an excellent response of acute tuberculous pneumonia treated with viomycin. The degree of clearing by roentgenogram is comparable to that usually seen with streptomycin and PAS therapy.

*Case 9* (H. W.). A 39-year-old Negro male building superintendent was admitted on January 25, 1951, complaining of fever and weight loss for ten days. The present illness began in the fall of 1948, when a painful swelling developed in the midthoracic region. Following admission to the Hospital for Joint Diseases, a diagnosis of Pott's abscess of the spine was made, and a spinal fusion from the eighth through the twelfth dorsal vertebrae was performed on December 8, 1948. At this time he was immobilized in a plaster jacket. Streptomycin, 1.0 gm. daily without any PAS, was given from September 7, 1948, until discharge

on February 11, 1949. In January, 1949, when a new jacket was applied, it was noted there were two sinuses present at the left posterior chest wall, draining moderate amounts of purulent material. These sinuses continued to drain up to the time of his admission to Bellevue Hospital in 1951. Several days before admission the patient became febrile, exhausted, and dyspneic. For twenty-four hours, right-sided pleuritic chest pain had been noted. The past history was relevant in that there has been spontaneous drainage of pus from a swollen cervical lymph node when the patient was in his early twenties. During the same period he had received treatment for syphilis.

*Physical examination:* The patient's rectal temperature was 99°F.; pulse rate per minute, 72; the respiratory rate was 18 per minute; and blood pressure, 120 systolic and 80 diastolic in mm. of mercury. The patient was a well-developed but poorly nourished Negro male who appeared acutely and chronically ill. Two closely placed sinuses over the left posterior chest at the level of the ninth and tenth ribs were draining copious amounts of pus, requiring the use of massive dressings and a Kelly pad. There were signs of fluid, namely, dullness and decreased breath sounds over the right lower chest. The remainder of the examination was essentially normal.

Figure 5 is a roentgenogram of the sinus tracts overlying the left posterior ninth and tenth ribs as outlined by Lipiodol instillation. The residual of the right-sided pleural effusion with blunting of the costophrenic angle is also apparent without any visible parenchymal involvement.

Roentgenograms of the bones demonstrated partial destruction of the ninth and tenth ribs posteriorly.

*Laboratory findings:* Tubercle bacilli were cultured from the pus draining from the sinus tracts. Thoracentesis (right) yielded 350 cc. of thin yellow fluid, with specific gravity of 1.024, leukocyte count 1,700 per cu. mm., with a differential count of 75 per cent polymorphonuclears and 25 per cent lymphocytes. The smears and cultures of the fluid were sterile. The remainder of the laboratory tests yielded values which were within normal limits.

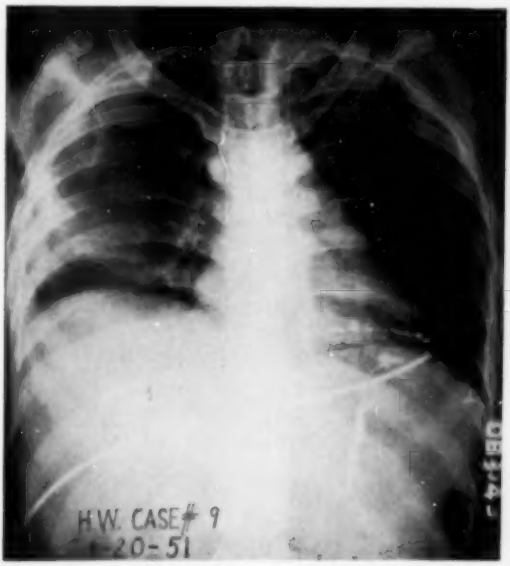


FIG. 5. *Case 9* (H. W.)
Roentgenogram of the sinus tracts outlined by Lipiodol

FIG. 6A

FIG. 6B

FIG. 7

*Course:* Viomycin therapy was instituted on March 22, 1951, in dosage of 3 gm. daily for 91 days. Up to this time there had been no change in the amount of daily drainage from the two sinus tracts. The pleural effusion had resorbed spontaneously, leaving an obliterated right costophrenic angle. The patient had remained chronically ill and had failed to gain weight on bed rest alone. His temperature had ranged between low fever and normal. After only two weeks of viomycin therapy, there was a notable decrease in the amount of drainage from the sinuses, and within four weeks dressings were reduced to 4- by 4-inch gauze pads. The patient lost his feeling of malaise, remained afebrile, improved in his general sense of well-being, and gained a total of 11 pounds during viomycin therapy. Figures 6*A* and *B* are photographs of the sinus tracts before viomycin therapy was started and their condition at the end of the first month of treatment. Although the sinuses were not completely epithelialized at the end of therapy, there was no drainage during the last month of viomycin treatment. A surgical consultant advised against resection of the ninth and tenth ribs. The patient left the hospital against advice on June 21, 1951, because he felt completely well.

This is a striking instance of closure of draining cutaneous sinuses from a Pott's abscess under viomycin therapy in a patient whose tubercle bacilli had become streptomycin resistant.

*Case 17* (L. C.)[4]. A 55-year-old Italian married bartender was admitted on March 4, 1950, complaining of weakness for six months. During this period there had been a total weight loss of 45 pounds. A chronic cough productive of one cupful of mucopurulent sputum daily had been present for two months, accompanied by fever and night sweats. Hoarseness without sore throat or dysphagia was noted for two months.

*Physical examination:* The patient's rectal temperature was 100.6°F.; pulse rate per minute, 84; the respiratory rate was 20 per minute; and blood pressure, 106 systolic and 70 diastolic in mm. of mercury. The patient was well developed but poorly nourished and appeared chronically ill. He was raising large amounts of purulent sputum. Indirect laryngoscopy showed redness and swelling of the epiglottis and thickening of both vocal cords, with marked injection but no ulceration. There was no lymphadenopathy. The chest was symmetrical, with fair bilateral expansion and slight increase in anteroposterior diameter. There was moderate dullness over the upper third of the left lung, with bronchovesicular breath sounds, increased vocal fremitus, and numerous fine and medium moist rales. A few post-tussic rales were audible at the right apex and infraclavicular region.

Admission chest roentgenogram was interpreted as showing caseocavitary disease involving the entire left upper lobe with some bronchogenic spread below. The upper half of the right lung field contained small exudative and caseous infiltrates with areas of honeycombing.

*Laboratory findings:* The sputum was positive for tubercle bacilli on smear and culture. The remainder of the laboratory studies revealed no abnormalities.

*Course:* The patient received daily combined streptomycin and PAS therapy, 1 gm. and 9 gm., respectively, for 91 days, ending June 13, 1950. His symptomatic response was excellent. Tubercle bacilli cultured from his sputum after the eighth week of treatment,

[4] The reconstructive neck surgery in this case was performed by Dr. John F. Daly, Professor and Chairman, Department of Otolaryngology, New York University Post-Graduate Medical School.

FIG. 6. *Case 9* (H. W.)

*A*. Colored photograph of the draining tuberculous sinus before viomycin was instituted.
*B*. Colored photograph of the sinus after one month's treatment with viomycin.

FIG. 7. *Case 17* (L. C.)

Colored photograph of the final condition of the laryngectomy wound

however, became resistant to streptomycin in concentration of 100 γ per ml., and this resistance persisted. Despite some degree of roentgenographic clearing bilaterally, the laryngeal symptoms did not improve.

Direct laryngoscopy in November, 1950, revealed fixation of the left vocal cord. A biopsy revealed a squamous cell carcinoma of the larynx. Combined streptomycin and PAS therapy was reinstituted immediately, and laryngectomy was performed on January 4, 1951.

The neck wound became involved with a horrible, sloughing, tuberculous mixed infection, and a large granulating fistula developed into the pharynx. Examination of tissue obtained by biopsy of the wound revealed tuberculosis. Tubercle bacilli were cultured from the wound and found to be resistant to streptomycin in concentration of 100 γ per ml. The patient failed to made any progress toward healing the neck wound during the six months following his operation.

Because of the seriousness of the patient's condition, viomycin therapy was instituted on July 1, 1951, in a dosage of 3 gm. daily. After the first week of viomycin therapy the tuberculous cellulitis of the neck began to improve dramatically. The patient's general condition also gradually improved, with increased strength, excellent appetite, a weight gain of 12 pounds in the first three months, and diminished cough and expectoration. After two months of therapy, examination of the neck wound showed it to be completely healed. The pharyngostome was covered with stratified squamous epithelium on the external surface and with mucous membrane on the inner surface. There was a redundant mass of esophageal mucosa present just above the tracheal stoma. After 101 days of daily viomycin therapy, the dosage was changed to 3 gm. twice weekly, except for 50 days of daily viomycin during the operative period of plastic reconstruction of the pharynx from December, 1951 to early January, 1952. Thereafter, viomycin was given twice weekly until March 22, 1952.

Repair of the laryngectomy wound and plastic reconstruction of the pharynx were accomplished by the use of the esophageal and pharyngeal mucosa which was redundant at the site of the pharyngostome, reinforcing this with sliding grafts from the neck. The condition of the flaps was excellent, and the laryngeal stoma was an extremely wide one. Since this would not contract down, it was possible to dispense with the tracheotomy tube. This

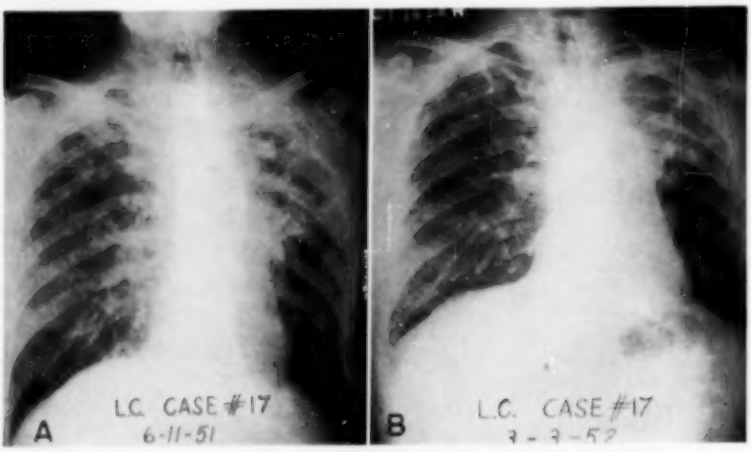


FIG. 8. *Case 17* (L. C.)

*A*. Roentgenogram dated June 11, 1951, prior to institution of viomycin therapy.

*B*. Roentgenogram dated March 3, 1952, after eight months of viomycin therapy, prior to thoracoplasty.

represented a very satisfactory end to a very difficult and trying situation. A photograph of the final condition of the neck is shown in figure 7.

The only evidence of toxicity to viomycin exhibited by this patient was a slight loss of hearing in the high frequency tones on serial audiograms.

After a total of eight months of viomycin therapy, the chest roentgenograms had shown moderate bilateral clearing (figures 8*A* and *B*). The patient's general clinical condition was excellent. Tracheal aspirations of secretions remained positive for tubercle bacilli on culture but negative on smear. Since cavitary disease in the left upper lobe persisted, it was considered advisable to do a left seven-rib thoracoplasty. A first-stage thoracoplasty was performed on March 19, 1952, uneventfully. As the susceptibility of the patient's tubercle bacilli to viomycin was not known at this time, the treatment was arbitrarily changed to isoniazid on March 22, 1952. He received 300 mg. daily and underwent a second-stage thoracoplasty on April 2, 1952. The postoperative course was uneventful, and cultures of tracheal aspirates have remained negative for tubercle bacilli. On November 5, 1952, the patient was transferred to a sanatorium for another six months of bed rest before starting rehabilitation.

In this case the therapeutic effect of viomycin was life-saving in a patient with advanced bilateral pulmonary tuberculosis and a post-laryngectomy tuberculous cellulitis of the neck, since the tubercle bacilli were streptomycin resistant. Plastic neck surgery and eventual thoracoplasty were possible under viomycin therapy.

## DISCUSSION

In the clinical evaluation of a new antituberculous drug, the investigator may choose the method of analyzing data from a large number of cases or he may obtain his information from a careful study of fewer selected patients, basing his judgment largely on extensive previous experience with tuberculosis. Each approach has advantages and limitations. The present writers were interested in conducting a limited study as carefully and intensively as possible, and the facilities and patients available lent themselves to this purpose. Accordingly, reliance was placed, not only on rather precise data which would be suitable for statistical analysis, but also on a number of familiar clinical observations which, although important, cannot be interpreted so clearly. An effort was made to identify the many features of each case and to consider their possible meaning.

Patients were chosen for viomycin treatment because of certain lesions from which they suffered, especially if the disease had failed to respond previously to standard forms of treatment or seemed unlikely to respond. Most of the lesions were pulmonary, but a number of others were in other tissues. Other antimicrobial therapy had been given in some but not in all of the cases; if it failed, this may have been due to bacterial resistance to the drugs or to the character of the disease. Anticipating from the studies of others that the antimicrobial effects of viomycin would not differ essentially from those of streptomycin, attention was directed mainly to a consideration of the comparative efficacy and toxicity of viomycin. Attention was also focused on the important ability of this drug to inhibit the growth of tubercle bacilli refractory to the inhibitory effects of streptomycin, for this might provide an alternative means of treatment.

It may be seen from table 1 that the case material was extremely varied. No difference was noted between daily and intermittent drug regimens, in regard to either therapeutic effect or drug toxicity.

While treatment, with or without drugs, often produces favorable general and symptomatic effects, it is well known that simultaneous changes in local lesions are most striking when these are still in an inflammatory, exudative phase without much destruction of tissue. Chronic caseocavitary pulmonary lesions, for instance, may "dry up" and shrink somewhat, but the early change is not commensurate with the resolution observed in early pneumonic lesions. In this respect, viomycin was not found to differ from other antituberculous drugs in its effects on the various types of lesion. Even though the pulmonary lesions of some patients were not expected to be influenced materially by the drug, some patients were included nevertheless because an effect might be observed in an extrapulmonary lesion. Obviously, therefore, while such local responses were noted and they added information of value, the rating of degree of over-all improvement may have been low. A drug may be a potent antimycobacterial agent but, once this effect has been exerted, the power of the body to heal a lesion depends as well on other factors. These may be adequate in one case but not in another; or of varying adequacy for different lesions in the same patient.

It is worth noting that there were very few patients (*Cases 5, 12, 19, 22,* and *23*) who showed local progression of their disease while receiving viomycin. Only one had a spread of disease to uninvolved portions of the lung, and this followed bronchoscopy (*Case 18*). Unfortunately, the susceptibility of the tubercle bacilli to viomycin was not known in this instance.

Prior to the advent of potent antituberculous drugs, there were certain serious complications of advanced pulmonary tuberculosis which all too frequently heralded the end of a patient's course. Reference is made to those lesions caused by mucosal surface contamination with mucopus heavily laden with tubercle bacilli, e.g., tuberculous laryngitis, endobronchitis, and enteritis. As seen from the cases briefly presented in table 1, these complications did not appear as new lesions during treatment in this series and, when they existed beforehand, they proved amenable to viomycin therapy in a manner comparable to that observed with other antituberculous drugs. The usefulness of viomycin as a potent drug against tuberculosis is well exemplified in the draining sinus cases.

The clinical response of the 23 patients treated with viomycin was fairly striking, even compared with rather similar cases exhibiting clinical response to streptomycin or isoniazid, which, weight for weight, clearly have a greater quantitative antituberculous effect. Eighteen of the 23 patients became afebrile. There was appreciable weight gain in 17 and reduction of sputum volume in 17. Sputum "conversion" to negative for tubercle bacilli by culture was infrequent, however, being shown by only 4 of the 23 patients.

The toxicity of viomycin is appreciable and caution in its use is undoubtedly necessary, with the performance of frequent laboratory determinations, especially those needed to detect hypokalemia and nerve deafness. The therapeutic effect of viomycin is great enough to warrant its use in patients harboring tubercle bacilli resistant to other drugs. It is possible that intermittent dosage regimens will avoid or decrease the manifestations of toxicity of viomycin without lessening its therapeutic usefulness in selected cases.

The present studies of resistance of tubercle bacilli to viomycin are insufficient for interpretation, but others (9) have demonstrated the occurrence of viomycin-resistant strains.

It is possible that regimens combining viomycin with another drug, such as streptomycin or PAS, will delay emergence of bacterial resistance to viomycin (10). This deserves further study. It is the impression of the present writers that the degree of efficacy of viomycin is less than that of streptomycin or isoniazid but greater than that of PAS.


## SUMMARY

The therapeutic effect of viomycin in a series of 23 cases of human tuberculosis is presented. The patients were treated with drug regimens of 50 to 60 mg. per kg. of body weight, given daily or on a twice weekly schedule, for periods of 30 to 263 days. Far advanced pulmonary tuberculosis was present in 21 patients, and extrapulmonary tuberculosis was demonstrated in 9 of the 23 patients. Eleven patients were known to have tubercle bacilli resistant to streptomycin, and 8 patients had never received previous antimicrobial therapy.

It is evident from both the tabulated results and the representative case reports that viomycin had appreciable antituberculous action in this series of patients. Viomycin has definite but not prohibitive toxicity. Laboratory facilities, chiefly audiometry and plasma electrolyte studies, should be available if viomycin is to be safely administered.

Further studies of the emergence of bacterial resistance to viomycin are indicated. Additional studies using intermittent and combined drug regimens seem warranted.

Viomycin apparently is less effective against human tuberculosis than streptomycin or isoniazid. Therefore, the chief indication for viomycin therapy appears to be in treatment of patients with tubercle bacilli resistant to the latter two drugs who require additional chemotherapy.



## SUMARIO

### *La Viomicinoterapia en la Tuberculosis Humana*

Descríbese el efecto terapéutico de la viomicina en una serie de 23 casos de tuberculosis humana. Los enfermos fueron tratados con regímenes medicamentosos de 50 a 60 mg. por kg. de peso, administrados en esquemas ya diarios o de dos veces semanales durante períodos de 30 a 263 días. Había tuberculosis pulmonar muy avanzada en 21, y se descubrió tuberculosis extrapulmonar en 9, de los 23 enfermos. Según se averiguó, 11 enfermos tenían bacilos tuberculosos resistentes a la estreptomicina y 8 jamás habían recibido antes antibióticoterapia.

A juzgar tanto por los resultados tabulados como por típicas historias clínicas, es manifiesto que la viomicina ejerció apreciable efecto tuberculostático en esta serie. La viomicina posee toxicidad bien definida, pero no prohibitoria de su uso. Para administrar viomicina inocuamente, debe haber a mano medios de laboratorio, en particular para ejecutar estudios audiométricos y de los electrolitos del plasma.

Están indicados nuevos estudios de la formación de resistencia bacteriana a la viomicina. Parecen además justificados estudios en que se utilicen regímenes medicamentosos intermitentes y combinados.

La viomicina es aparentemente menos eficaz que la estreptomicina o la isoniacida contra

la tuberculosis humana. Por lo tanto, la principal indicación de la viomicinoterapia parece radicar en el tratamiento de pacientes que tienen bacilos tuberculosos resistentes a las últimas dos drogas y necesitan más farmacoterapia.


## RESUME

*La thérapie par la Viomycine dans la tuberculose humaine*

Présentation des résultats thérapeutiques obtenus avec la viomycine dans une série de 23 cas de tuberculose humaine. La posologie, pour les malades en traitement, était 50 à 60 mg. par kilogramme de poids corporel administrés chaque jour ou deux fois par semaine pendant une période variant entre 30 et 263 jours. Chez 21 malades la tuberculose pulmonaire était au stade avancé, chez 9 malades il existait en outre des signes de tuberculose extra-pulmonaire. Onze malades étaient reconnus comme porteurs de bacilles tuberculeux streptomycino-résistants. Huit malades n'avaient jamais été traités par les antibiotiques.

Il est évident, comme le montrent les résultats figurant dans les tableaux et dans les rapports des cas qui s'y rapportent que, dans cette série, la viomycine a fait preuve d'une activité tuberculostatique appréciable. La viomycine possède une toxicité définie, mais non prohibitive. Les procédés de laboratoire permettant d'effectuer certains examens, surtout l'audiométrie et la détermination des électrolytes plasmatiques, doivent être accessibles si on veut administrer la viomycine avec sécurité.

Des études complémentaires sur l'apparition de la résistance à la viomycine des bactéries sont indiquées. Des études additionnelles sur la thérapeutique comportant l'administration discontinue de la viomycine, ou son association à d'autres substances, paraissent justifiées.

Il semble que la viomycine soit moins efficace que la streptomycine ou l'isoniazide dans la tuberculose humaine. En conséquence, l'indication principale de la viomycinothérapie paraît devoir être le traitement des malades porteurs de bacilles tuberculeux résistants à ces deux antibiotiques et pour lesquels il y a lieu de pratiquer un traitement supplémentaire.


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# THE USE OF VIOMYCIN IN PATIENTS WITH PULMONARY TUBERCULOSIS[1, 2]

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## INTRODUCTION

Viomycin,[5] an antimicrobial produced by a species of *Streptomyces*, was first described in 1951 (1, 2). It is unusual among this class of antimicrobial agents in that its biologic activity is virtually restricted to the mycobacteria (1, 2, 3). The drug has been shown to be effective in the treatment of experimental tuberculosis in both mice (4, 5) and guinea pigs (6, 7). Animal studies (8) further indicated a relatively low degree of toxicity, although cats did develop a vestibular disturbance similar to that seen with streptomycin. One report (9) has appeared regarding the use of viomycin in 10 patients with far advanced pulmonary tuberculosis. In this report emphasis was placed on the toxic manifestations, since evaluation of the chemotherapeutic response was difficult in the patients studied. The dosage which was used in these patients varied from 30 to 75 mg. per kg. per day, and the duration of treatment ranged from 14 to 182 days. Albuminuria of some degree was found almost universally, and this was accompanied by the presence of casts and erythrocytes in the urine. Diminished vestibular function and some hearing loss were noted in 2 of the patients. A curious type of toxic manifestation associated with electrolyte imbalance was noted in 5 patients. This consisted of a fall in the concentration of serum potassium, calcium, phosphorus, and chlorides and an increase in the carbon dioxide combining power of the serum. The electrolyte changes were discovered after 2 patients had developed clinical tetany. The remaining 3 patients suffered only from paresthesias and muscle pain.

The effect of viomycin in clinical tuberculosis has been under further investigation since the fall of 1950. The combined experience of a number of investigators was presented to the Tenth Streptomycin Conference in January, 1951, by Muschenheim (10) and consisted of a report of 49 cases. It was the opinion of the investigators at that time that the drug had definite beneficial effect in clinical tuberculosis and that continuation of the investigation was indicated. In 1953, Hobby (11) summarized the evaluation of the use of viomycin in 375 patients. It was concluded that this agent was valuable in the treatment of tuberculosis when other less toxic drugs were ineffective or for some reason could not be used.

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[5] Viomycin was kindly supplied by Dr. Gladys L. Hobby of Chas. Pfizer & Co., Inc. Brooklyn, New York

Efforts have been made to reduce the toxic effects of viomycin by its use on intermittent dosage schedules. There are indications that with such regimens there is continued therapeutic effectiveness (12) and that disturbances of serum electrolytes and of renal function are negligible (13).

The present report concerns the therapeutic results obtained in 12 patients treated with viomycin for pulmonary tuberculosis.

## PLAN OF INVESTIGATION

### *Selection of Patients*

Although all patients selected (*see* table 2) had advanced pulmonary tuberculosis, those with extensive, predominantly bilateral disease who had little immediate prospect of undergoing definitive therapy were not included as a general rule. Two major criteria were met as closely as possible. First, patients were selected who were known to be, or were inferred to be, harboring streptomycin-resistant tubercle bacilli. Secondly, the patients had had an acute exudative spread of disease following surgery or had well-localized chronic disease but with an acute exudative "spread." In either case the acute disease threatened life or compromised the possibility of definitive surgical therapy directed at the chronic focus of disease. In several instances, the acute spread of disease which was treated with viomycin had occurred during streptomycin therapy. It was reasoned that selection of such patients offered an ethical basis for the use of a drug of definite toxic potential in which therapeutic effectiveness had been incompletely evaluated and represented situations in which one would be willing to accept the risk of some toxicity. The majority of these patients had received several prolonged courses of streptomycin previously.

## METHODS

Viomycin dosage varied from 40 to 60 mg. per kg. per day for periods ranging from 7 to 244 days. The dosage and duration of therapy in each individual patient are given in the toxicity table (table 1). Dry viomycin sulfate was diluted in distilled water to a concentration of 0.4 gm. per ml., and the total daily dosage was divided into two portions and given morning and evening. The dosage was continued for a period of at least 120 days in 7 of the 12 patients, although in 2 cases it was interrupted for a short period of time by the appearance of a generalized pruritic skin eruption. In these cases it was possible to reinstitute therapy with the administration of antihistaminic drugs and by desensitization procedures. The remaining 5 patients, who received less than 120 days of viomycin therapy, had the drug discontinued because of toxicity, sensitivity reactions, or because of death from tuberculosis.

Studies aimed at the protection of the patients from severe toxicity included weekly routine urinalysis; complete blood count monthly; urea clearance and blood urea nitrogen at intervals of two weeks; serum potassium and sodium determinations at weekly intervals; blood calcium, phosphorus, and carbon dioxide combining power determinations at monthly intervals; monthly bromsulfalein tests of liver function and electrocardiograms at irregular intervals in a few patients. Audiometric studies and vestibular function studies by means of caloric stimulation were obtained in the majority of these patients, but the vestibular function tests were impossible to evaluate since alterations had occurred previously in most subjects owing to prior streptomycin therapy.

## OBSERVATIONS

### *Toxic Reactions*

*Local:* Only one patient (*Case 7*) had significant local reaction at the sites of injection of the viomycin sulfate employed. Local tenderness and erythema necessitated brief discontinuation of the drug on the ninety-third day of treatment.

*Drug hypersensitivity:* Hypersensitivity to viomycin manifested as a generalized urticarial, erythematous, or maculopapular skin eruption led to discontinuation of therapy in 4 instances (*Cases 6*, *8*, *10*, and *11*). Two patients (*Cases 6* and *10*) experienced urticaria, one had a maculopapular rash (*Case 11*), and the fourth had an erythematous rash. One patient with urticaria (*Case 10*) and one with an erythematous rash (*Case 8*) were successfully desensitized and continued therapy without recurrence. The skin eruptions appeared between the seventh and twenty-third days of viomycin therapy. Antihistaminics were effective in controlling pruritus in each instance. One patient (*Case 11*) experienced beginning exfoliative dermatitis on the seventh day of viomycin therapy.

*Renal:* Albuminuria, varying from a trace to 3-plus, occurred in 8 of the 12 patients. This was transient in all but one (*Case 10*) who had persistent albuminuria during the 244-day course of viomycin. The albuminuria disappeared within one month after cessation of therapy. Very occasional granular casts were present in the urinary sediment of these same 8 patients. One patient (*Case 5*) had some microscopic hematuria which had been present prior to the reception of viomycin and which disappeared while he received viomycin. A few leukocytes were observed in the urine of 3 patients, but 2 had lower urinary tract infections. No reducing substance was detected in the urine. No elevation of blood urea nitrogen occurred.

*Hepatic:* There was no evidence of hepatotoxicity of viomycin. One patient (*Case 3*) had approximately 10 per cent retention of bromsulfalein before, during, and after therapy. No change occurred in the total serum proteins of any patient.

*Neurologic:* Tinnitus occurred in 4 patients, with no audiometric evidence of loss in auditory acuity in one (*Case 3*), but as a prodrome or concomitant of hearing loss in the remaining 3 (*Cases 7*, *10*, and *12*). The loss of auditory acuity was slight in *Cases 7* and *10* and, although it was deemed advisable to discontinue viomycin in *Case 7* because of hearing loss after 99 days of therapy, viomycin was continued for 244 days in *Case 10*, although slight loss in auditory acuity was demonstrable after 120 days of treatment. Loss of hearing in *Case 12*, on the thirty-sixth day of treatment, was sudden, with tinnitus appearing only thirty-six hours earlier. A return of some of the loss within ninety days after cessation of viomycin was gratifying. No other neurologic disorders were observed.

*Electrolyte:* No significant alteration in serum electrolytes was observed. These patients did not receive adjuvant electrolyte intake.

### *Clinical Course*

Of the 12 patients presented, 7 were thought to have significant amounts of exudative pulmonary tuberculosis to observe roentgenographically for evidence

TABLE 1

VIOMYCIN TOXICITY

| NUMBER | PATIENT | DURATION OF THERAPY | DOSAGE | | | TOXICITY | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Gm. per Day | Mg. per Kg. | Total Gm. | Hepatic | Renal (Albuminuria) | Hypersensitivity | Ototoxicity |
| 1 | M. M. | 120 | 2.4 | 60 | 288 | 0 | Trace to 3+ within 3 weeks | 0 | 0 (prior streptomycin loss) |
| 2 | P. L. | 120 | 2.7 | 60 | 324 | 0 | Trace to 1+; only 2 occasions after 30 days | 0 | 0 (prior streptomycin loss) |
| 3 | D. M. | 48<br>72<br>120 | 3.3<br>2.6 | 50<br>40 | 159<br>187<br>346 | 0 | 0 | 0 | Tinnitus (mild; transient) |
| 4 | L. D. | 35<br>85<br>120 | 2.4<br>1.6 | 60<br>40 | 84<br>136<br>220 | 0 | 1+ from fourth to eighth weeks | 0 | 0 |
| 5 | J. W. | 120 | 2.8 | 40 | 336 | 0 | Trace; 1 occasion | 0 | 0 |
| 6 | M. S. | 23 | 2.4 | 40 | 55 | 0 | 0 | Generalized urticarial skin rash on twenty-third day | 0 (prior streptomycin loss) |
| 7 | R. K. | 99 | 2.4 | 40 | 238 | 0 | Trace to 2+ on 2 occasions | 0 | Tinnitus and slight acuity loss after 80 days |
| 8 | D. B. | 120<br>120<br>240 | 2.0<br>2.0 | 40<br>40 | 240<br>240<br>480 | 0 | 0 | Generalized erythematous skin rash: on seventeenth day of first course; on ninth day of second course (desensitized) | 0 |
| 9 | E. R. | 36 | 2.4 | 40 | 86 | 0 | Trace; 1 occasion | 0 | 0 |
| 10 | G. S. | 244 | 2.4 | 40 | 586 | 0 | Trace to 3+ throughout therapy | Generalized urticarial skin rash on thirteenth day (desensitized) | Tinnitus with slight acuity loss after 4 months. No progression. |
| 11 | L. S. | 7 | 2.0 | 40 | 14 | 0 | 0 | Exfoliative dermatitis of hands on seventh day | 0 (prior streptomycin loss) |
| 12 | J. G. | 36 | 3.0 | 40 | 108 | 0 | 1 to 3+ after 3 weeks of therapy | 0 | Tinnitus on thirty-fourth day; marked acuity loss on thirty-sixth day |

of benefit from viomycin therapy (*Cases 1, 2, 4, 5, 8, 10,* and *12*). Two patients had tuberculous bronchitis which precluded their reception of definitive surgical therapy (*Cases 3* and *8*). Of the remaining 4, one had extensive pulmonary fibrosis (*Case 7*) and 3 had such extensive pulmonary involvement (*Cases 6, 9,* and *11*) that viomycin was employed only in an attempt at salvage.

TABLE 2

RESULTS OF VIOMYCIN THERAPY

| PATIENT, SEX, AGE, RACE | EXTENT AND DURATION OF TUBERCULOUS DISEASE | TYPE OF TUBERCULOUS DISEASE TREATED | DURATION OF THERAPY | RESPONSE |
|---|---|---|---|---|
| 1. M. M., F, 36, W | Far advanced bilateral, 2½ years<br>Left thoracoplasty (2 years) | Exudative; postoperative pneumonectomy | 120 | Marked |
| 2. P. L., M, 20, W | Far advanced bilateral, 3 years<br>Previous PNX and PPN | Fibrocavernous<br>Exudative; postoperative thoracoplasty | 120 | Moderate<br>Moderate |
| 3. D. M., M, 31, W | Far advanced bilateral, 1 year<br>Progressive disease | Fibrocavernous<br>Bronchitis | 120 | Slight<br>Moderate |
| 4. L. D., F, 37, W | Far advanced bilateral, 3 years<br>Left lower lobe bronchiectasis | Exudative; postoperative lobectomy | 120 | Marked |
| 5. J. W., M, 41, W | Far advanced bilateral, 9 years<br>Previous bilateral PNX | Caseocavernous; exudative | 120 | Moderate |
| 6. M. S., F, 40, N | Far advanced, right, 2½ years<br>Pott's disease | Fibrocavernous; exudative | 23 | None |
| 7. R. K., M, 28, W | Moderately advanced bilateral, 2 months<br>Sarcoidosis, 4 years | Sarcoidosis; exudative | 99 | Slight |
| 8. D. B., F, 29, W | Moderately advanced bilateral, 1½ years<br>Progressive disease | Exudative; cavernous;<br>Bronchitis<br>Caseocavernous; exudative | 120<br><br>120 | Moderate<br>Marked<br>Slight |
| 9. E. R., F, 65, W | Far advanced bilateral, 4 years<br>Prior right middle and lower lobectomies (4 years) | Caseocavernous; exudative | 36 | None |
| 10. G. S., M, 48, W | Far advanced bilateral, 10 years<br>Prior left PNX | Exudative<br>Empyema (tuberculous) | 244 | Moderate<br>— |
| 11. L. S., F, 47, W | Far advanced bilateral, 2 years<br>Right thoracoplasty (6 months) | Fibrocavernous<br>Exudative | 7 | —<br>— |
| 12. J. G., M, 46, W | Moderately advanced, right, 17 years<br>Right PNX (17 years)<br>Right thoracoplasty (1 year) | Exudative; postoperative lobectomy | 36 | Moderate |

PNX = therapeutic pneumothorax.
PPN = therapeutic pneumoperitoneum.

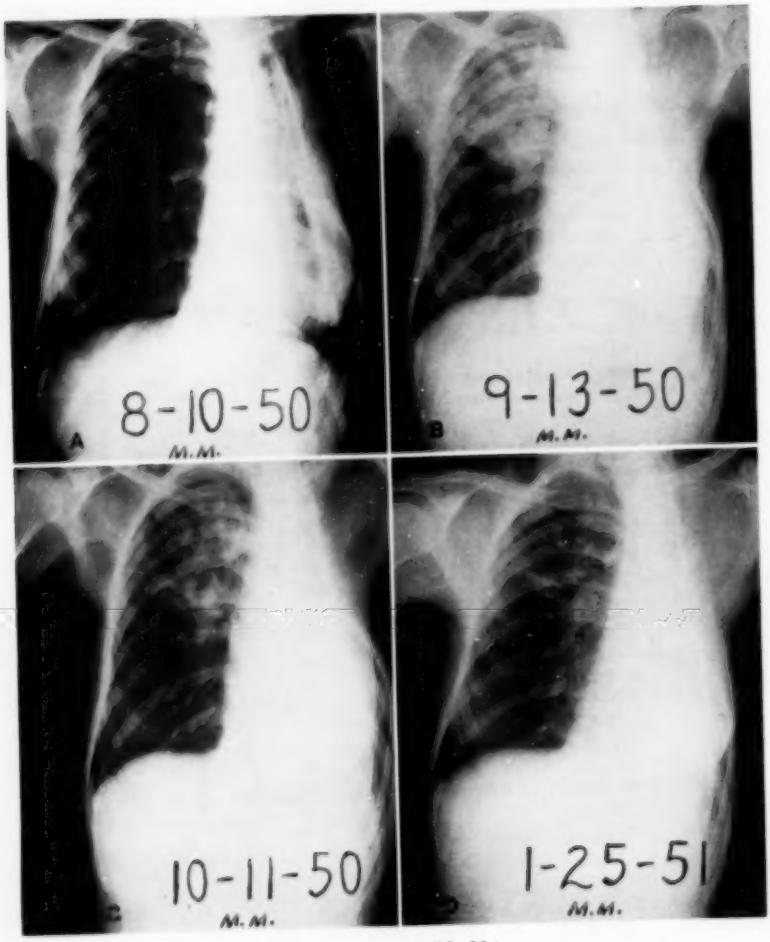


FIG. 1. *Case 1* (M. M.)

*A*. This 36-year-old white female had moderately advanced pulmonary tuberculosis in the left upper lung since 1948. A left thoracoplasty was performed in 1948, but the sputum remained positive for tubercle bacilli following several courses of streptomycin. She entered University Hospital on August 8, 1950. No disease on the right.

*B*. A left pneumonectomy was done on August 28, 1950. Spread of tuberculous disease to the right lung occurred while streptomycin and para-aminosalicylic acid were given. Viomycin was started on September 6, 1950.

*C*. Following treatment with viomycin for one month, marked improvement of the right upper lung disease has occurred.

*D*. After three months of viomycin therapy there is continued clearing of the disease.

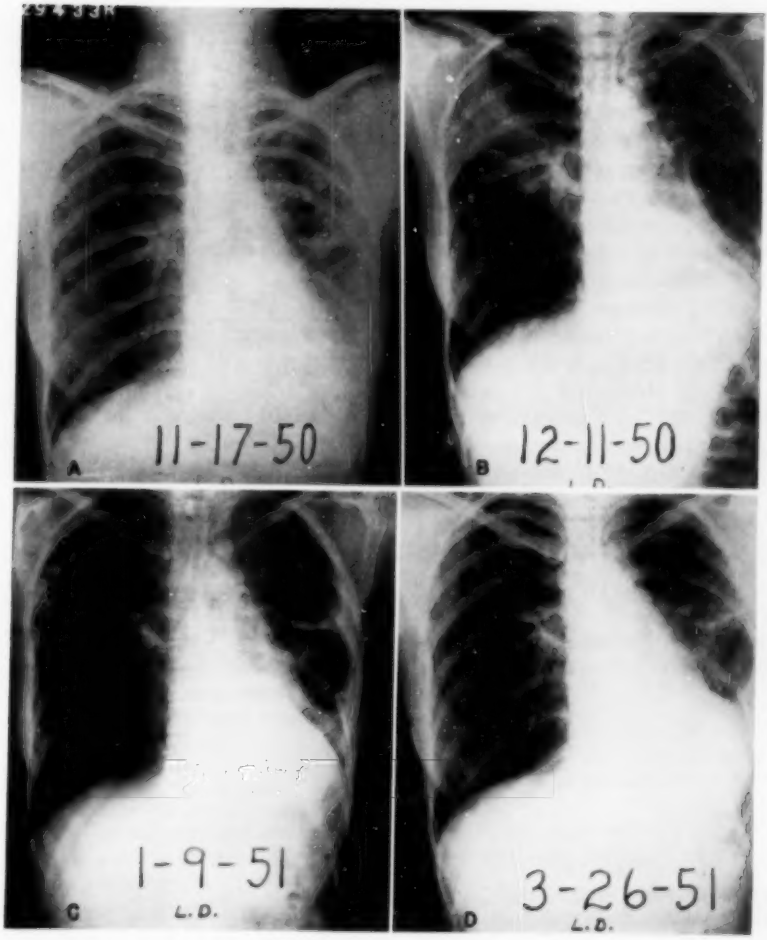


FIG. 2. *Case 4* (L. D.)

*A*. This 37-year-old white female had moderately advanced tuberculosis in 1947 and received streptomycin, left phrenic crush, and pneumoperitoneum. Sputum remained positive for tubercle bacilli, and removal of a bronchiectatic left lower lobe was done in September, 1950. The immediate postoperative course was satisfactory, and the right lung remained free of disease.

*B*. In December, 1950, spread of tuberculous disease occurred on the right, and viomycin was started on December 13, 1950.

*C*. After one month of viomycin therapy there was marked improvement in the right-sided disease.

*D*. Further clearing of the disease occurred during the next two months of viomycin treatment.

In table 2, a description of the extent and type of tuberculous disease and the observed clinical and roentgenographic response to therapy are presented.

Of the 7 patients with primarily exudative disease, there was marked response of this exudative disease to viomycin therapy in 2 instances (*Cases 1* and *4*). The course in these 2 patients illustrates (figure 1, *A–D*; figure 2, *A–D*) both the optimal type of pulmonary disease to treat with a recently available antituberculous agent and the response which would indicate the antituberculous activity of the agent. The 5 remaining patients showed moderate roentgenographic improvement (*Cases 2*, *5*, *8*, *10*, and *12*). No patient had progression of tuberculous disease while receiving viomycin.

The 2 patients with tuberculous bronchitis (*Cases 3* and *8*) had very satisfactory bronchoscopic evidence of healing, which in one patient was nearly complete (*Case 8*). The latter patient subsequently was able to have a left pneumonectomy for control of disease.

The 4 "salvage" patients were either not treated sufficiently long (*Cases 6*, *9*, and *11*) or, in one instance, evaluation was precluded by pulmonary fibrosis (*Case 7*). Clinical improvement did occur, however, in *Case 7*, and sputum cultures became negative for *M. tuberculosis*.

Eight of the 12 patients, followed twenty-two to thirty-six months after cessation of viomycin therapy, are living. One has been discharged from the hospital with inactive tuberculosis (*Case 5*). Seven remain hospitalized, but 3 have sputum cultures negative for tubercle bacilli. Two of the 4 deaths followed progressive tuberculous disease after viomycin was discontinued. One patient (*Case 11*) received only seven days of viomycin therapy. One of the remaining patients died from air embolism during a space-closing thoracoplasty following pneumonectomy, and the other (*Case 9*) died two days following a first-stage thoracoplasty. The latter received only thirty-six days of viomycin therapy.

## SUMMARY

Twelve patients with pulmonary tuberculosis have been treated with viomycin sulfate in a daily dosage of 40 to 60 mg. per kg. per day for periods of 7 to 244 days. Attempts were made to choose patients with recently acquired, primarily exudative tuberculous disease, resulting from progressive disease or postoperative spread of disease in whom the tubercle bacilli involved were proved or presumed to be resistant to streptomycin.

Toxic manifestations of viomycin therapy occurred. The loss of auditory acuity, which appeared in 3 patients, was of greatest concern. This loss was slight in 2 patients, but acute and nearly total in the third. Significant return in auditory acuity occurred in the third patient three months after viomycin was discontinued. Hypersensitivity reactions with urticarial, erythematous, or maculopapular skin eruptions occurred in 4 patients. Desensitization was possible in 2 patients. No electrolyte imbalance was noted. Renal toxicity as manifested by albuminuria and cylindruria was common but transient. No hepatotoxicity was observed.

Clinical and roentgenographic improvement was most marked in those pa-

tients with recently acquired exudative tuberculous disease. Improvement was marked in 2 and moderate in 5 of the 7 patients who had such pulmonary disease. Tuberculous bronchitis improved in 2 patients. In 2 instances (*Cases 10* and *12*) viomycin was thought to be life saving. Eight of the 12 patients are alive twenty-two to thirty-six months after receiving viomycin therapy.

Viomycin does exert a useful chemotherapeutic effect in pulmonary tuberculosis, although it is unquestionably less active than streptomycin. It is believed that further study of the antituberculous activity of viomycin is justified and indicated.

There undoubtedly will be fewer patients in whom this drug is indicated since the advent of isoniazid, but situations will continue to arise in which it should be used. In an effort to decrease or possibly preclude toxic effects of the drug, it should be employed on an intermittent treatment schedule and undoubtedly in combination with other antituberculous drugs.


## SUMARIO

*El Empleo de la Viomicina en los Tuberculosos Pulmonares*

Doce enfermos con tuberculosis pulmonar han sido tratados con sulfato de viomicina a dosis diarias de 40 a 60 mg. por kg. durante un período de 7 a 244 días. Tratóse de escoger a enfermos que tenían afección tuberculosa primariamente exudativa, recién adquirida y proveniente de enfermedad evolutiva o difusión postoperatoria, en quienes los bacilos tuberculosos en acción habían resultado, o se suponía que eran, resistentes a la estreptomicina.

Hubo manifestaciones tóxicas de la viomicinoterapia. La pérdida de la agudeza auditiva, que afectó a 3 enfermos, fué la que ocasionó más preocupación. Esa pérdida fué ligera en 2 enfermos, pero aguda y casi total en el tercero. En éste, hubo retorno importante de la agudeza auditiva a los tres meses de discontinuarse la viomicina. En 4 enfermos aparecieron reacciones de hipersensibilidad, con erupciones cutáneas urticáricas, eritematosas o maculopapulares. La desensibilización resultó posible en 2 de ellos. No se notó desequilibrio electrolítico. La intoxicación renal, traducida por albuminuria y cilindruria, fué común, pero passajera. No se observó hepatotoxicidad.

La mejoría clínica y radiográfica fué más notable en los enfermos que tenían afección tuberculosa exudativa recién adquirida. La mejoría fué acentuada en 2 y moderada en 5 de los 7 enfermos que tenían enfermedad pulmonar de dicho género. La bronquitis tuberculosa mejoró en 2 sujetos. En 2 casos (10 y 12), la viomicina pareció salvar la vida. Ocho de los enfermos están vivos de veintidós a treinta y seis meses después de recibir la viomicinoterapia.

La viomicina ejerce en verdad un útil efecto quimioterapéutico en la tuberculosis pulmonar, aunque es sin duda menos activa que la estreptomicina. Parece que está justificado e indicado un estudio ulterior de la actividad antituberculosa de la droga.

Induddablemente habrá menos enfermos en quienes esté indicada esa droga desde el advenimiento de la isoniacida, pero continuarán surgiendo ocasiones en que deba usarse. Con mira a atenuar o posiblemente evitar los efectos tóxicos de la viomicina, debe emplearse en un esquema de tratamiento intermitente e incuestionablemente en combinación con otras drogas antituberculosas.

## RESUME

*Emploi de la Viomycine chez les malades atteints de tuberculose pulmonaire*

Douze malades atteints de tuberculose pulmonaire ont été traités par le sulfate de viomycine à la dose de 40 à 60 mg. par kg. par jour pendant une période de 7 à 244 jours. Les

sujets qui ont été choisis de préférence présentaient des formes surtout exudatives et récentes résultant de la dissémination de la tuberculose soit post-opératoire soit par progression de l'affection, et chez lesquels la streptomycino-résistance des bacilles tuberculeux intéressés était démontrée ou probable.

Des manifestations d'intolérance à la viomycinothérapie se sont manifestées; la perte de l'acuité auditive, qui s'est produite chez trois malades, était la plus importante. Cette déficience fut légère chez deux malades, mais aiguë et presque totale chez le troisième. Une récupération importante de l'acuité auditive s'est manifestée chez le troisième malade trois après la suppression de la viomycine. Des réactions allergiques avec urticaire, érythème ou lésions papulo-maculaires de la peau se sont produites chez quatre malades. La désensibilisation a pu être réalisée chez deux sujets. Il n'a été observé aucune altération de l'équilibre des électrolytes. Les manifestations de toxicité rénale, albuminurie et cylindrurie, furent fréquentes, mais transitoires. Il n'a été observé aucun signe de toxicité hépatique.

L'amélioration clinique et radiologique fut des plus marquée dans les cas de tuberculose exsudative récemment acquise. L'amélioration a été marquée chez deux malades et modérée chez cinq malades sur sept présentant cette forme de tuberculose pulmonaire. La bronchite tuberculeuse s'est améliorée chez deux malades. Dans deux cas (Nos 10 et 12), la survie du malade a été attribuée à la viomycine. Huit de ces douze malades sont vivants vingt-deux à trente-six mois après la viomycinothérapie.

La viomycine exerce une action thérapeutique utile dans la tuberculose pulmonaire, bien qu'elle soit indubitablement moins active que la streptomycine. La poursuite des recherches sur l'activité antituberculeuse de la viomycine est justifiée et indiquée.

Le nombre de malades chez lesquels cette médication est indiquée sera sans doute moins élevé dans l'avenir en raison de l'apparition de l'isoniazide, mais il y aura encore des cas où elle devre être utilisée. Dans le but de réduire, ou peut-être de prévenir ses effets toxiques, l'administration de la viomycine devra être intermittente et incontestablement conjuguée à d'autres médicaments antituberculeux.

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# INDICATIONS FOR SEGMENTAL RESECTION IN PULMONARY TUBERCULOSIS[1, 2]

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## INTRODUCTION

The role played by segmental resection in the over-all treatment of pulmonary tuberculosis has gone through a period of development similar to other surgical procedures which have been introduced for the control of this disease. Since 1946 the present writers have used this operation in the treatment of pulmonary tuberculosis; first, in a limited way, and gradually with widening indications for its employment. During this period, the writers have developed certain definite concepts concerning the use of this surgical procedure, and it is believed that in certain instances the indications for segmental resection are sharp and clear cut. In a number of cases, however, there is some question whether or not segmental resection will prove to be the procedure of choice.

The present experience consists of a series of 121 cases of pulmonary tuberculosis in which segmental (or localized) resection of tuberculous lesions was performed. The medical literature pertaining to segmental resection in pulmonary tuberculosis has not been extensive, and the majority of the articles have stressed operative technique. Therefore, a discussion of the indications for segmental resection in pulmonary tuberculosis in the light of experience gained in this series is presented.

The term "segmental resection" is now being used for the anatomic excision of a segment of the lung, as well as the simple excision of subsegments or portions of a segment of the lung. Strictly speaking, a "segmental resection" should mean the complete removal of the pulmonary segment by the anatomic approach as first reported by Churchill and Belsey (1) and later modified by Blades (2), Clagett and Deterling (3), Overholt and Langer (4), and Chamberlain and Klopstock (5). There are many instances, however, in which the amount of diseased tissue in the lung is less than one whole segment, so that it is unnecessary and undesirable to remove the entire pulmonary segment. The decision rests mainly upon anatomic considerations, the amount of pulmonary tissue which is actively diseased, the type of involvement, and upon physiologic and technical problems.

The writers believe that for practical purposes resection of total segments,

[1] The patients were treated in the San Fernando Veterans Hospital and in private hospitals in Los Angeles.

[2] Presented, in part, before the Medical Session, as part of the session on *Surgery in the Treatment of Tuberculosis*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, May 19, 1953.

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subsegments, or localized resection of lung tissue should be considered together. If less than one entire lobe of the lung is removed, it is impractical to consider in separate categories varying amounts of lung tissue resected in less than one segment.

In this series, no attempt has been made to perform excision of tiny quiescent tuberculous lesions which have not completely disappeared from the roentgenogram following antimicrobial therapy, or to excise every tiny pulmonary nodule in the lung which was discovered at operation. In the majority of the cases, the resection has included one or more segments of the lung.

## BASIC OBJECTIVES OF PULMONARY RESECTION

The basic objective of segmental resection in pulmonary tuberculosis is the excision of the actively diseased portions of the lung with the maximum conservation of normal functioning pulmonary tissue. Thus, segmental resection will find its greatest effectiveness in early and moderately advanced pulmonary tuberculosis. In this situation, it should be possible to remove all of the active tuberculous lesions without the need of extensive ancillary collapse measures.

In far advanced pulmonary tuberculosis, segmental resection can be employed in a limited number of cases to remove the cavitary lesions, relying on the effective control of the caseous and noncavitary active disease by bed rest, chemotherapy, and secondary collapse measures.

Segmental resection is particularly advisable in the cases in which, because of the low pulmonary reserve, it is essential to conserve functioning pulmonary tissue. The following conditions may greatly lower the pulmonary reserve of the patient:

Contralateral collapse of the lung
Contralateral resection of the lung
Contralateral tuberculous disease
Pulmonary emphysema
Pulmonary fibrosis
Pleural disease

Thus, when pulmonary function is seriously limited by the above conditions, segmental resection could seriously be considered. Nevertheless, the number of such cases in which segmental resection may actually be performed effectively is limited by the fact that all too often the cavitary disease is not limited to one or more resectable segments, even with extensive use of secondary collapse procedures. It is unfortunate that frequently the patients who, from a physiologic standpoint, need segmental resection the most are those in whom the disease is so extensive as to preclude the use of the procedure.

## PROPER TIME FOR SEGMENTAL RESECTION

Segmental resection is performed most successfully when the acute phase of the tuberculosis has subsided, the disease process has been stabilized, and the patient has been able to mobilize a maximum resistance against the tubercle bacillus.

Thus, the patient should be a "good chronic," a term used at one time to describe the patient who was considered to represent an ideal candidate for thoracoplasty.

The introduction of antimicrobial therapy has greatly reduced the duration of the period of bed rest necessary to stabilize the disease and build up the maximum resistance. All of the patients in this series, except the earlier ones, have had chemotherapy and bed rest prior to surgery, with the exception of those with diagnostic problems. It is believed that the use of long-term chemotherapy with the use of streptomycin, para-aminosalicylic acid (PAS), or isoniazid, in pairs or all together, has greatly improved the results.

It is known that the present antimicrobial drugs are not eradicative in action but are most effective in controlling exudative lesions and, presumably in some instances, prevent any further necrosis. It is also known that antimicrobial drugs exert little effect on fibrous lesions per se, although the repair of fibrous tissue may be stimulated by control of the tuberculous lesion. Nevertheless, the capacity of the antimicrobial drugs to inhibit the proliferation of the bacilli in the diseased lung provides an opportunity for the reparative processes of the body to function most effectively.

When streptomycin was introduced, the rapid emergence of strains of drug-resistant tubercle bacilli limited the widespread administration of the drug. Thus, streptomycin was saved for crises in the course of the disease (6), such as acute exudative spread, and for protection following surgical procedures. However, with the use of multiple chemotherapy with streptomycin, para-aminosalicylic acid and, more recently, isoniazid, it is now possible to administer some form of chemotherapy at virtually any stage of a patient's tuberculosis.

The beneficial effects of the concurrent use of streptomycin and PAS are well known. More recently, experimental evidence of the added value of isoniazid has led the writers to include this drug in the long-term schedule of chemotherapy. Although final proof is not available at this time, it appears that tubercle bacilli disappear from the sputum more rapidly with the triple-drug regimen.

The regimen in use at present consists of streptomycin, 1.0 gm. two times per week; PAS, 12 gm. per day; and isoniazid, 150 mg. per day, for long-term courses.

It is the practice to institute drug therapy with bed rest as soon as a cure of the patient is started in cases of exudative disease in which the lesion is acute or subacute. Thus, the forward progress of the disease is stopped and fatal spread or irreparable pulmonary damage is prevented in most cases. The drugs are of lesser value in chronic fibrotic disease, which is static. However, they are used in these cases as a preoperative preparation of the patient to lessen, if possible, the number of tubercle bacilli in the fibrotic cavities.

Patients with acute exudative disease are given a period of three to six months of antimicrobial therapy. At these periods, the cases are reviewed for the suitability of segmental resection. This represents a marked departure from the almost universal former therapeutic practice when, without chemotherapy, the patient was given from six months to a year or more of bed rest before any surgical procedure was considered. At the present time, in addition to bed rest and chemotherapy, some form of collapse therapy may be indicated in the moderate and far advanced cases.

Pneumothorax, the once time-honored method of collapse which was used in practically all cases, is now being gradually replaced. This is due to the fact that this procedure is not as effective as was once thought and is fraught with very definite complications. The present writers have not abandoned it completely, as has been the case in many medical centers in the country, but believe that its use is very limited.

Pneumoperitoneum has proved effectual in controlling mid-lung exudative disease and is now used with much greater frequency than pneumothorax. It has no effect on fibrous disease and rarely closes the cavity present in lesions predominantly of this type.

Because it is possible to raise the diaphragm with pneumoperitoneum, phrenemphraxis has been used very little in the present series before operation.

Thoracoplasty has been employed in a few instances as a primary collapse procedure when primary segmental resection was not indicated. However, the failure of thoracoplasty to close the cavities and control the disease occurs mostly in the more advanced forms of pulmonary tuberculosis, for which a subsequent lobectomy or even pneumonectomy will be necessary, and not a segmental resection. The use of these secondary collapse measures must be considered in patients with moderately and far advanced disease, however, for in many instances the parenchymal tuberculous disease can be sufficiently controlled to permit the segmental resection of the offending uncontrolled tuberculous cavity.

## TUBERCULOUS LESIONS SUITABLE FOR SEGMENTAL RESECTION

In table 1 the list of tuberculous pulmonary lesions suitable for segmental resection are tabulated.

TABLE 1

TUBERCULOUS LESIONS SUITABLE FOR SEGMENTAL RESECTION

| | |
|---|---|
| I. Absolute indications (not controlled by collapse) | |
| | Blocked cavities |
| | Thick-walled cavities |
| | Tension cavities |
| | Middle and lower lobe cavities |
| | Atelectasis |
| | Bronchiectasis |
| | Tuberculomas |
| II. Diagnostic problems | |
| III. Elective indications | |
| | Simple cavities (also controlled by collapse) |
| | Tension cavities (rarely controlled by collapse) |
| | ? "potentially dangerous" caseous or fibrocaseous lesions |

*Absolute indications:* By "absolute indications" are meant those situations in which the disease is obviously not amenable to control by collapse therapy. Segmental resection is a method of choice in localized active tuberculous lesions which fall into this category. Blocked cavities are well known to be resistant to

collapse measures. If they are sufficiently localized, it is believed that their presence is an important indication for primary segmental resection.

It is difficult to collapse thick-walled cavities effectively, even by thoracoplasty, so that primary excision of these lesions is indicated. When there is considerable fibrous tissue about the walls of a cavity, however, most often there is extensive pulmonary disease, so there are only a few cases in which segmental resection is technically possible.

Tension cavities most often resist collapse because of a check-valve obstruction of the bronchus which permits the trapping of air. It is true that this check-valve mechanism depends upon the bronchocavitary relationship, and this may be so altered in some instances by thoracoplastic collapse as to permit the bronchus to drain normally and the collapse to become effective. In these circumstances, resection is much more certain than collapse, however, and may save the patient both time and additional surgical procedures.

Middle and lower lobe cavities are almost invariably resistant to pulmonary collapse and, if localized in one segment, are best treated by segmental resection.

Atelectasis of the lung containing active tuberculous disease usually results from bronchial obstruction and is more effectively managed by resection than by thoracoplastic collapse. Since bronchiectasis is uninfluenced by collapse, primary segmental resection is indicated.

Tuberculomas are also unaffected by collapse measures so that resection is the only satisfactory method of handling this type of disease. Although it is generally agreed that enlarging tuberculomas and the larger lesions which are often indistinguishable from blocked cavities should be removed, the indications for the excision of the smaller and apparently quiescent tuberculomas are still unsettled.

*Diagnostic problems:* The appearance of a roentgenographic shadow which may represent a potentially dangerous lesion to the patient with minimal or absent symptoms presents a situation which is always trying to both physician and patient. This is particularly difficult in the older age groups in which the possibility of bronchogenic carcinoma makes an exploratory operation urgent. In these circumstances, it is distinctly unwise to wait for a number of months for the gastric lavage specimen to be found positive or negative for tubercle bacilli by guinea pig inoculation or culture. Moreover, the presence of a positive tuberculin test is not too helpful in such situations although, if the tuberculin reaction is negative, the indication for operation is all the more urgent.

The problem is further complicated by the fact that it has been shown that a bronchogenic carcinoma may smoulder along with imperceptible growth for months or even a year or more until some unknown factor changes the growth potential of this lesion and widespread dissemination results. Certainly, the best results in cancer surgery have been in the early asymptomatic or relatively asymptomatic lesions.

*Elective indications:* Simple cavities which persist following chemotherapy with antimicrobials and bed rest are now considered indications for segmental resection, providing they are sufficiently localized and the exudative disease has been controlled. There is no question that many of these patients can be cured by

thoracoplasty. Such excellent results have been obtained with the resection of the localized upper lobe cavity, however, that the writers are beginning to prefer it to thoracoplasty. Certainly there is less deformity and the patient is subject to a single operation. In carefully selected cases, it is believed that the percentage of "sputum conversion" will be greater and the time spent on bed rest therapy will be less than with thoracoplasty. Although, as indicated above, some tension cavities can be managed by collapse therapy, their resection offers a more certain result.

The problem of what to do with the small caseous or fibrocaseous lesion of the lung which persists following chemotherapy in the patient who is clinically well, and whose sputum no longer contains tubercle bacilli, is indeed a difficult one.

Medlar (7) has stressed the point that small lesions in the superior and posterior portions of the pulmonary lobes tend to have a necrotic focus which sloughs into the connecting bronchus. Lesions in the other areas of the lung less often produce sloughing. He believes it is the persistence of unhealed necrotic pneumonic tuberculous lesions which cause relapses of disease at a later date in persons who appear to be clinically well. This is the rationale for resection of the small lesion, as carried out by Ryan, Medlar, and Welles (8). The present writers believe that long-term studies will be necessary before the value of localized pulmonary resection has been definitely established.

Certainly, from the theoretical point of view, the eradication of these lesions is desirable. Some of them do break down at a later date. It is not yet established, however, how many unnecessary operations will be performed if this practice is widely followed. The writers do believe, however, that study of the presence of tubercle bacilli and their viability is urgently necessary in the resected specimen of lung. Furthermore, long-term follow-up studies on similar cases treated medically are also indicated. Time should bring an answer to this problem.

## REQUIREMENTS FOR SEGMENTAL RESECTION

Certain minimal requirements must be met if segmental resection is to prove an effective procedure in the treatment of pulmonary tuberculosis. As has been stated before, the active disease must be limited to one or more resectable segments. The resected lesion should be the only one which prevents the complete healing of the operated lung.

In developing the segmental fissure, the surgeon must not cross active disease located in the fissure line because failure of healing can be anticipated. No potentially active disease must remain in the operated lung. It is believed that overdistension of the remaining portions of the lung tends to reactivate latent tuberculous disease. Hence, it is the practice of the writers to attempt to avoid over-all distension at all costs. Such overdistension can be prevented by pneumonolysis of the lung with complete severance of all of its adhesions, freeing the lobes, developing interlobar fissures, if possible, and the division of the inferior pulmonary ligament. This permits the lung to expand and turn on its axis a bit so that it can more completely fill the pleural space. Moreover, decortication of pleural membranes from adjacent portions of the lobe and other lobes will permit

maximum expansion of the lung and filling in of the dead space created by the segmental resection.

If these two procedures are not effective in obliterating the dead space, then secondary collapse measures are indicated. Most often the writers have used pneumoperitoneum with crushing of the phrenic nerve at the diaphragm to provide a more selective action of the pneumoperitoneum. The air is introduced into the peritoneal cavity through a stab wound with a purse-string suture, used for closure.

Refills are given as often as necessary following the operation. By crushing the phrenic nerve at the diaphragm, early return of diaphragmatic function can be anticipated. The length of continuance of pneumoperitoneum following the operation depends entirely upon the amount of disease present in the lungs before operation and the type of disease.

In some instances, when it has been considered inadvisable to perform either a phrenic crushing or a pneumoperitoneum, a localized modified upper thoracoplasty has been performed. This is best done at the time of surgery when the collapse can be "tailor-made" to fill in the dead space actually created by the pulmonary resection. The lung must fill the pleural cavity at time of closure of the thorax, and air which leaks out from the fissure line must be removed by gentle suction on the intrapleural drainage tubes to keep the lung constantly expanded.

It has been found that patients with extensive pulmonary fibrosis and emphysema in the adjacent lung segments and lobes have the poorest result with segmental resection. They are most in need of secondary collapse measures so that there is absolutely no strain of expansion on the remaining portions of the lung following segmental resection.

Contralateral disease is not a definite contraindication providing that the contralateral disease can be brought under control following the segmental resection. If surgical collapse or pulmonary resection is anticipated for the contralateral lung, however, the clinicians must weigh very carefully the ultimate pulmonary reserve of the patient and the amount it will be impaired by the bilateral surgical treatment. One must be careful that bilateral surgery necessary to render the sputum negative for tubercle bacilli does not make the patient a respiratory cripple, chained to a bed or chair by his low pulmonary reserve.

## INDICATIONS IN PRESENT SERIES

The indication for segmental resection in the 121 patients (123 operations) treated by the present writers is shown in table 2.

It will be noted that the great majority of these patients listed in table 2 had active pulmonary tuberculosis. Open cavity was the greatest indication (37 cases) and represented, for the most part, failures of the medical regimen. Almost half of these might be considered elective cases in that thoracoplasty might well have closed the cavity.

In the group of blocked cavities (27 in number), the resection was an absolute indication, for collapse would fail in this type of lesion. Practically all of the

TABLE 2

INDICATIONS FOR SEGMENTAL RESECTION IN PULMONARY TUBERCULOSIS

| NATURE OF DISEASE | NUMBER OF OPERATIONS |
|---|---|
| Open cavity | 37 |
| Blocked cavity | 27 |
| Active fibrocavernous and caseous | 20 |
| Diagnostic problems | 19 |
| Tuberculomas | 8 |
| Decortication and active lesions | 8 |
| Bronchiectasis | 2 |
| Atelectasis | 2 |

patients had had sputum intermittently positive for tubercle bacilli on serial examinations, although practically all of them, when operated upon, had sputum negative for tubercle bacilli, presumably as a consequence of chemotherapy.

The group of 20 active fibrocavernous and caseous lesions was composed of a variety of pathologic conditions which showed up for the most part as small cavities on the planigrams and of larger caseous lesions with intermittent discharge of tubercle bacilli. These have been grouped in one heading as it is impossible to consider so many small categories on an individual basis.

In 19 cases, thoractomy was performed because of suspicion of bronchogenic carcinoma. The patients were all in the cancer-age group and had persistent or enlarging peripheral shadows on the roentgenogram. All of these patients had sputum negative for tubercle bacilli and, at surgery, a tuberculoma of the lung was the common finding. The group of 8 patients with tuberculomas represents a younger age group with tubercle bacilli in the sputum whose roentgenograms showed lesions believed to be quite characteristic of tuberculoma. These were circumscribed lesions frequently with the typical concentric laminations. Some of them showed calcium deposits. All except 2 of these 8 patients were positive reactors to tuberculin.

In 8 cases, segmental resection was carried out secondary to a pulmonary decortication. It is believed that this is an important indication for segmental resection because, with the thorax open, the surgeon has an excellent opportunity to remove all active or potentially active tuberculous lesions which are frequently hidden by the thick pleural membrane. If expansion of the lung is effected by the decortication, then abnormal tension on these lesions may lead to their reactivation. Resection is preferable to leaving the lesion unmolested, even if the pleural membrane is also left as a protective covering over the lesion.

Segmental bronchiectasis, resulting in definite symptomatology, was the indication in 2 instances; while atelectatic segments of the lung, with the intermittent discharge of tubercle bacilli, were indications in 2 other cases.

## POSTOPERATIVE COMPLICATIONS

Tabulation of the postoperative complications is presented in table 3.

In 3 instances, extensive postoperative intrapleural hemorrhage occurred.

TABLE 3

COMPLICATIONS IN 121 CASES OF SEGMENTAL RESECTION

| COMPLICATION | NUMBER OF PATIENTS |
|---|---|
| Postoperative intrapleural hemorrhage | 3 |
| Postoperative spread of tuberculosis | 4* |
| Bronchopleural fistula | 2 |
| Wound infection | 1 |
| Postoperative atelectasis | 1 |
| Empyema | 2 |

* Healed with drugs.

In one of these cases, it was possible to evacuate the blood and clot through the intercostal tubes with the use of streptokinase and streptodornase. In the 2 other cases, it was necessary to reopen the chest and to stop the hemorrhage occurring from multiple tiny capillaries on the parietal pleura at the site of extensive intrapleural adhesions. The hemorrhage was controlled by using mattress sutures with muscle grafts and with the actual cautery. In both patients, the bleeding and clotting time were normal before surgery although they showed this definite tendency to abnormal capillary bleeding.

Postoperative spread of tuberculosis occurred in 4 instances, but was controlled by chemotherapy.

Bronchopleural fistula developed in 2 patients. In both instances, far advanced disease was present and, in the light of present knowledge, the 2 cases represented an attempt to extend the indications for segmental resection to disease which was too far advanced.

There was transient leaking of air in a number of cases, presumably from the intersegmental fissure. This was controlled by the continuation of the use of gentle suction on the intrapleural drainage tubes which had been applied immediately after surgery.

Wound infection, present in one case, responded to drainage; while postoperative atelectasis and empyema were each present in one instance. These last three complications did not result in significant morbidity to the patient.

There were 2 postoperative deaths. The first occurred in a 67-year-old male who presented a diagnostic problem compatible with bronchogenic carcinoma. He died from a myocardial infarction one week after operation. The second death occurred in a 48-year-old female who had a persistent pulmonary cavity following unsuccessful thoracoplasty. She died as a result of a pulmonary embolism on the fourth day after a resectional operation.

## FOLLOW-UP MANAGEMENT AND OBSERVATIONS

*Follow-up management:* Following operation, it is the practice to give these patients care similar to that for persons who have undergone lobectomy and pneumonectomy for pulmonary tuberculosis (9). In segmental resection, greater diligence must be exercised to effect immediate and sustained expansion of the

lung. As tuberculosis is a disease which usually affects both lungs, even in microscopic amounts, it cannot be assumed that segmental resection has removed all of the tuberculous disease in the lungs. For this reason, the patients have been kept on a regimen of modified bed rest and chemotherapy for a minimal period of three months, an average of six months and, in some instances, up to one year or more following operation.

Only one temporary reactivation has been observed in the patients so treated. In some instances, the pneumoperitoneum has been continued for one year, although this is uncommon. At present the writers believe that chemotherapy should be given to patients with preoperative sputum positive for tubercle bacilli for a minimum of six months following surgery, and that nine months to one year may be the optimum period for administration of these drugs.

*Follow-up observations:* As most of the patients in the present series have been operated upon in the last year and a half, long-time follow-up studies are not available. All of the patients operated upon earlier are well, but this represents, for the most part, a group in which conservative indications for surgery were employed. At the present time, only 5 patients have sputum positive for tubercle bacilli, and these are patients recently operated upon. Early "conversion" of the sputum in these cases is anticipated. Certainly, the early clinical results in the present series of patients have been excellent.

## GENERAL COMMENT

Segmental resection is coming to occupy an important position in the surgical treatment of pulmonary tuberculosis. It has its greatest field of usefulness in early and moderately advanced disease. With early diagnosis, it is anticipated that more patients will be considered suitable for pulmonary resection. Such early diagnosis can be accomplished by mass roentgenographic surveys and similar routine examinations of persons admitted to general hospitals, and by a general educational program to alert the patient and the practicing physician to the problems of early diagnosis. With the increased use of such measures, the present availability of resectional procedures offers considerable hope for the future control of tuberculosis.

If the indications and requirements for segmental resection which have been outlined in this paper are rigidly adhered to, it is believed that this form of treatment is ideal because it conserves pulmonary tissue; makes the arrest of the disease permanent; offers minimal thoracic cage deformity; and effects this with minimal expenditure of time for the patient.

It seems to the writers that, in instances of active disease and open cavities, the indications, as outlined, have proved quite satisfactory. However, in the case of the small residual pulmonary lesion in a patient who has had chemotherapy and is apparently well, we believe there is much to be learned. The ultimate disposition of these lesions will depend upon long-term follow-up studies in operative and nonoperative cases.

SUMMARY

The indications for segmental resection in pulmonary tuberculosis, based on the experience of 121 operative cases, are presented.

Strict adherence to the requirements for operation which have been presented is necessary if satisfactory results are to be obtained. Poor results may be anticipated if the indications are extended to cases which are too far advanced.

The early results in this series have proved quite satisfactory although long-term follow-up reports are not yet available.

The number of postoperative complications has been low, and the operative mortality is 1.8 per cent.

Although the indications for segmental resection in active and cavitary pulmonary tuberculosis are fairly clear cut at this time, a final opinion cannot be given regarding the treatment of tiny persistent asymptomatic tuberculous lesions in the lung when sputum is negative for *M. tuberculosis*.

SUMARIO

*Indicaciones de la Resección Segmentaria en la Tuberculosis Pulmonar*

Expónense las indicaciones de la resección segmentaria en la tuberculosis pulmonar, a base de lo observado en 121 casos operados.

Para poder obtener resultados satisfactorios, hay que conformarse estrictamente a los requisitos operatorios aquí expuestos. Son de esperar malos resultados si se extienden las indicaciones a casos que están demasiado avanzados.

En esta serie, los resultados tempranos son bastante satisfactorios, aunque no se cuenta todavía con datos a largo plazo.

La proporción de complicaciones postoperatorias ha sido baja y la mortalidad operatoria es de 1.8 por ciento.

Si bien las indicaciones de la resección segmentaria en la tuberculosis pulmonar activa y excavada están bastante bien definidas actualmente, no cabe expresar una opinión definitiva acerca del tratamiento de las minúsculas y persistentes lesiones tuberculosas asintomáticas del pulmón cuando el esputo es negativo para el *M. tuberculosis*.

RESUME

*Indications de la résection segmentaire dans la tuberculose pulmonaire*

Présentation des indications de la résection segmentaire dans la tuberculose pulmonaire fondées sur l'expérience acquise au cours de 121 cas opérés.

Il y a lieu de se conformer strictement aux conditions spécifiées pour l'opération pour obtenir des résultats satisfaisants. Il y a lieu de s'attendre à des échecs si les indications opératoires sont étendues aux cas trop avancés.

Dans cette série les résultats initiaux se sont montrés satisfaisants, toutefois, les observations à long terme font actuellement défaut.

Le nombre des complications post-opératoires est faible et la mortalité opératoire est de 1,8%.

Bien que les indications de la résection segmentaire dans la tuberculose pulmonaire active et cavitaire soient assez bien établies maintenant, il n'est pas possible d'exprimer une opinion définitive en ce qui concerne le traitement des petites lésions tuberculeuses chroniques et asymptomatiques du poumon quand il n'y a pas de B.K. dans les crachats.

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# DESCRIPTIVE CLASSIFICATION OF PULMONARY SHADOWS[1]

## A Revelation of Unreliability in the Roentgenographic Diagnosis of Tuberculosis

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### INTRODUCTION

In 1946, a group of investigators, including some of the present writers (1), showed that photofluorograms are as good as standard roentgenograms for the detection of pulmonary tuberculosis. In the course of this study, it was noted that film readers failed to agree dependably on the presence of a lesion or its state of activity. Following the publication of this study, a board was appointed by the United States Public Health Service to study further this matter of reader disagreement and to explore the possibility of a revision of the roentgenographic code (in *Diagnostic Standards*) of the National Tuberculosis Association.

The aims of the studies conducted under the auspices of this board were: to measure the reliability of roentgenographic diagnosis in the field of tuberculosis; to try to invent a classification of pulmonary lesions as seen on the roentgenograms which would be apt to give optimal reliability; and to measure and prove the validity of such a classification.

### DEFINITIONS

At the outset of the investigation, certain terms were defined as follows:

*Reliability:* Reliability is essentially dependability. It is the frequency with which the same roentgenographic density is called by the same class name. If a radiologist examines a set of films a second time and his diagnoses do not contradict the diagnoses made on the first examination, then he is reliable, according to this definition. If several radiologists report on the same series of films according to an agreed set of terms or symbols and it is found that they seldom disagree, the classification is considered to be reliable.

*Validity:* Validity is the measure of medical usefulness. It is the correlation between the class name or symbol (diagnosis) and the clinical and pathologic condition of the patient. If the several classes of roentgenographic diagnosis correlate closely with the status of the patient's lesion, e.g., "open" or "closed," slow or fulminating, arrested or reactivated, et cetera, then the classification has high validity.

Obviously a classification, however reliable, has no use unless it is valid. Likewise, a classification of low reliability is not worth attempting to validate.

*Reading of roentgenograms:* For the purposes of this essay, reading consists in looking at a series of roentgenograms and reporting on them. Proper radiologic diag-

[1] This research was begun in the United States Public Health Service and continued with its financial support. The opinions expressed are those of the authors and do not reflect any official policy of the U.S.P.H.S.

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nosis takes into consideration everything known about the patient. The present analysis is concerned only with what a radiologist sees in a single film.

*Agreement and disagreement:* Two different readers saying the same thing or different things concerning the same pulmonary shadow represent *agreement* or *disagreement*, respectively.

*Self-confirmation and self-contradiction:* One reader's saying the same thing or two different things on two separate readings concerning the same pulmonary shadow is designated *self-confirmation* or *self-contradiction*, respectively.

## Plan of Investigation

The present writers read many films separately and together to discover their reliability as revealed by disagreements and self-contradictions in film readings. After doing this for one code of film reading (classification), other codes were devised which were intended to be more objectively descriptive of what is seen in the film, and the present investigators measured their reliability in the same way.

The above-described procedures were concerned with the first two aims of the large-scale investigation, namely, reliability and classification. The third aim, validation, has been under study by another group of investigators for several years and some of the results obtained have already been published (2).

The original plan of the present study was as follows: Several hundred chest films would be classified according to the appearance of the roentgenogram. All of these cases would be studied minutely and followed faithfully for several years. An objective statistical study would then show the meaning of each roentgenographic classification in terms of development of tuberculosis. It was believed that in the end it should be possible to state the clinical significance of the roentgenographic evidences of pulmonary tuberculosis in quantitative terms, presumably as a numerical coefficient of correlation between every category of the roentgenographic classification and its clinical counterpart. Unfortunately, however, a reliable classification, or roentgenographic code, was never forthcoming. The mechanism for validation was available, but the data to be validated could not be obtained.

## Observations

The measured reliability of the investigators is reported according to four codes of classification:

1. According to the code of the National Tuberculosis Association: minimal, moderately advanced, far advanced; active, probably active, inactive.
2. Descriptive of the pathology of the lesion as inferred by the radiologists: soft, hard, fibrotic, cavity, nummular, et cetera.
3. More objective description, involving structure, i.e., smooth, spotted, honeycombed, as well as shape, sharpness, and density.
4. Estimate of activity, made along with the objective description but as an independent direct judgment, i.e., unstable, stable, no judgment possible.

For every shadow reported by any reader as possibly a tuberculous lesion, comparison was made with its designation by the other two readers. For each

series of films which was read twice, each reader's second reading was compared with his first.

There were a number of excursions up "blind alleys," such as having each reader make a regular clinical-roentgenographic report on each film. These did not seem productive of agreement and mostly were ill adapted to statistical analysis.

Although the number of readings used for comparison totals more than one thousand, the analysis is concerned with the performance of only three radiologists, a small sample indeed. A second team of three radiologists was organized so that the basis for conclusions would not be quite so narrow. They failed to find any more promising route than the ones explored by the present writers. One of the members of this second team has published two essays (3, 4) on the subject of reliability of film readings which are based in part on the experience of both teams (six radiologists).

The present report could end here. The writers tried to find a reliable classification of the appearance of tuberculous lesions on the roentgenogram and must report failure to do so. It seems wise, however, to describe the methods used and to present the data in enough detail so that others may judge for themselves how well the possibilities were exhausted. It is possible that a review of this experience might perhaps lead to a more hopeful route for further exploration of the problem.

## SERIES 0

From the files of the Public Health Service, Dr. Henry Zwerling (not a member of this team but a participant in other studies of the problem) selected 100 films, approximately half of which showed lesions, mostly rather small, and in some instances only questionably abnormal. The films were read by the present writers who met together for the purpose, but made the readings independently. On the following day they read the films again. The classifications (N.T.A. code) were: *minimal, moderately advanced, far advanced* with the additional designations of *active, probably active, inactive*. This procedure was similar to that used by Birkelo and his associates (1). The results were similar to the results in their study, namely, some disagreement concerning the presence of a possibly tuberculous lesion, and more serious disagreement concerning the state of activity of those lesions agreed upon as representing tuberculosis.

The reliability of seeing a possibly significant shadow has since been evaluated (6) in several researches, but the present investigation deals with this aspect only in passing.

The data from Series 0 are presented graphically in figure 1. The two readings by each of three readers on 100 films provided 1,200 pairings between readers. Of these, 106 pairings were eliminated because one reader thought that the film showed pulmonary disease but that it was not tuberculosis. Of the 600 comparisons of a reader's own first and second readings, 40 were dropped out for the same reason.

The agreement on *negative* was 80 per cent. This figure has little meaning for the present studies because it depends on the proportion of abnormal films put into a series. In a general survey in which films showing abnormalities are encountered in only 1 or 2 per cent, it is known that agreement on *negative* will run approximately 98 per cent.

Of the 163 pairings of agreed "positive" films, there were only 12 in disagree-

ment as to extent. Of those read *moderately advanced*, there were 6 pairs in agreement and 8 pairs which one reader designated as *minimal*. One film was read *far advanced* and that by only one reader, each of the others always calling it *moderately advanced*, thus giving 4 pairs in disagreement.

Comparing the reader's second reading with his own first reading of the same film, in addition to the single case of *far advanced*, there were 5 self-confirmations on *moderately advanced* with only 2 self-contradictions. Self-confirmation on *negative* was 89 per cent.

Obviously, the collection of films contained too few advanced lesions to give a dependable quantitative estimate of reliability of classification by this code.

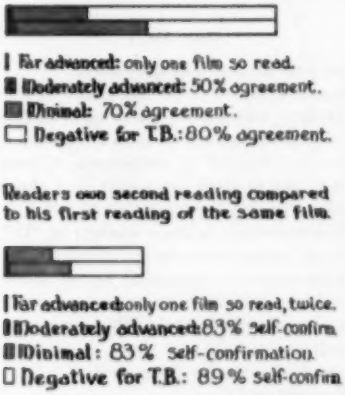
Far advanced: only one film so read.
Moderately advanced: 50% agreement.
Minimal: 70% agreement.
Negative for T.B.: 80% agreement.
Readers own second reading compared to his first reading of the same film.
Far advanced only one film so read, twice.
Moderately advanced: 83% self-confirm
Minimal: 83% self-confirmation
Negative for T.B.: 89% self-confirm

FIG. 1. Agreement by pairs, 100 selected films, read twice by three readers (1,200 pairings), according to the *Diagnostic Standards* of the National Tuberculosis Association. Self-confirmation, i.e., agreement of a reader's second reading compared to his first reading and *vice versa* (600 pairings). Whenever one reading was positive for disease, but nontuberculous disease or not in the lung, the pair was deleted.

As the extensional appearance of a lesion was not directly germane to the present study, no attempt was made to collect a larger series of advanced lesions.

In regard to estimate of activity, the disagreements were much greater, as had been true with Birkelo and associates (1). This is discussed subsequently at greater length. Only approximately one-half of the time did two readers agree definitely on *active* or *inactive*. Self-confirmation was only a little better. It was considered that there were two possible ways by which reliability might be improved.

1. Education and training with eventual gravitation of the three readers to a common basis. This did not work out, as will be seen when Series 3 is compared with Series 0 (*see* figure 7). Indeed, it had already been decided that such a possibility was not hopeful, for all of the three readers were radiologists of long experience, and yet were found to be unreliable in their own self-confirmations. If the reader could not agree with himself, with whom could he agree?

2. Improvement in the classification, i.e., substituting suitably chosen objective

descriptive terms for the presumably intuitive judgment: *active* or *inactive*. If terms could be found which had good linkage to the several varieties of shadows actually encountered, it should be possible to discover subsequently what clinical meaning to attach to each term. In essence, this is one of the original assumptions stated in the description of the plan of the present investigation (*vide supra*).

## SEARCH FOR A RELIABLE ROENTGENOGRAPHIC CLASSIFICATION OF PULMONARY LESIONS

At the first conference of the investigators, the following code was devised which was intended to provide symbols for nearly everything a radiologist might wish to say about the lesion.

*Code No. 1*

*Qualitative classification code for use with diagrams:*

| | |
|---|---|
| S | Consolidation. One or more airless lobules; parenchymatous infiltrate; soft lesion; patch of bronchopneumonia. |
| H | Hard lesion. Judged once to have been like "S," but subsequently became smaller by atelectasis, fibrosis, or calcification. Resolution per se does not result in a change which would be recognized as passing from "S" to "H." |
| F | Fibrotic strand. A linear variety of "H" so far shrunken as always to be judged completely obsolete. |
| N | Tuberculoma or nummular lesion. Round. |
| FS, FH, FSH | Combinations of above. |
| O | Cavity, air-filled. Fluid-filled cavity is "S" or "H" or "N." |
| Θ | Cavity with fluid and air. |
| R | Lymphadenopathy (lung root). |
| EFl | Encapsulated fluid. |
| KP | Not a pulmonary lesion, but in pleura. |
| KB | Not a pulmonary lesion, but in bone. |
| KM | Not a pulmonary lesion, but in chest wall or skin (muscle). |
| KV | Not a pulmonary lesion, but a vessel. |
| KT | Not tuberculous. |
| Ka | Artefact. |
| — or \ or / | Linear scar due to healed infarct or residual atelectasis. |
| "Q" | May be used if lesion cannot be defined. |

REMARKS: A question mark "?" may follow any code letter, e.g., H (?). Uncertainty between two designations may be expressed by adding the second in parentheses, e.g., S (or KB).

These symbols were to be written on a chart of the chest (figure 2) wherever the reader saw a significant shadow. He might use several symbols on the same lesion if he thought it composite in structure. While the investigators were still together, the use of the code was practiced to ensure that it meant the same thing to each of the group. Fifty new films were selected, showing a variety of simple and complex pulmonary lesions (possibly or presumably tuberculous). The collection was sent from one reader to another to be marked at leisure.

*Series 1:* In the 50 films there were 158 separate areas which one or another reader marked as a pulmonary lesion. Only 74 areas were so marked by all three

readers. Reader R. R. N. was alone in his designation of two lesions. Reader L. R. was alone for only one lesion. Reader W. E. C. was alone in his designation of 32 lesions, and tended to mark complex structure (triple symbols, *FHS*, in 25 per cent). At the other extreme was reader R. R. N., who used a single symbol in 76 per cent and triple symbols in none of the readings. These broad differences between readers subsequently diminished under the influence of group conferences, and it was decided that they were semantic rather than perceptual.

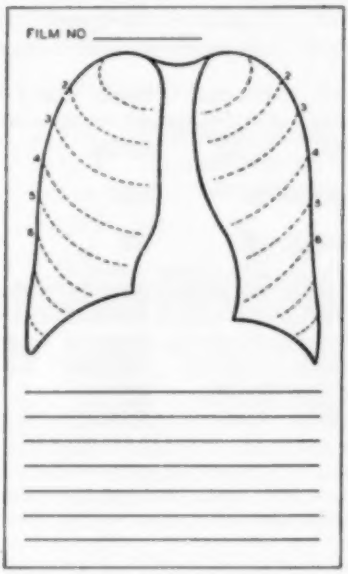


FIG. 2. Diagram of chest for first Code. Whenever he saw a lesion, the reader put a symbol, *S* for *soft*, *H* for *hard*, *F* for *fibrotic*, et cetera, according to the code given in the text. Symbols were available for nearly everything he might wish to say about a lesion, including denial that it was in the lung or belief that it was nontuberculous. He might mark the several parts of a lesion differently (compound symbols).

*Reliability under Code No. 1:* If agreement is defined *strictly*, any difference at all in the two readers' markings of the same lesion must be designated as disagreement even though both readers might easily be viewing the lesion as essentially the same. The disagreement, shown by adding an *H* (hard) to the marking, might be really quite trivial. It seemed wise, therefore, to analyze the data more generously, under an *inclusive* definition of agreement, in which agreement in part was accepted in spite of disagreement in part of a complex code marking. For this, each code letter was scored separately. Thus, a lesion marked *HF* by one and *HS* by another would be scored disagreement twice (once for *F* not agreed to, once for *S* not agreed to), and also scored agreement twice (once for one reader's *H* agreed to, once for the other reader's *H* agreed to). Pairs were accepted

only when both readers accepted the shadow as presumably tuberculosis. Some lesions, therefore, enter the analysis for one pair of readers and not for the other two pairs, because one reader thought the shadow not pulmonary, or non-tuberculous, or didn't see it at all.

The scores are shown graphically in figure 3. The readers agreed on the few cases of nummular lesion and in one case of unqualified cavity. If agreement is *strictly defined*, then 26 per cent of pairings were in agreement for the common types of lesion. Readers L. R. and R. R. N. averaged 55 per cent agreement. Agreement for reader W. E. C.'s pairings was injured by his inclination to complex markings, above noted. The average agreement was 40 per cent.

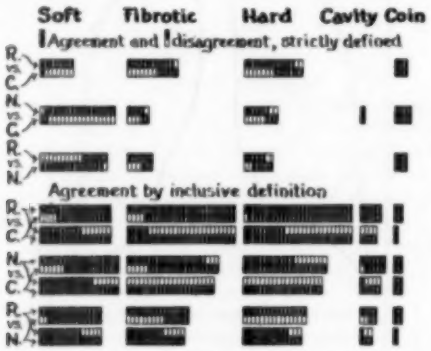


FIG. 3. Agreements and disagreements between readers. Only those pairings were used where both marked the shadow as presumably tuberculous. *Cavity* and *nummular* (coin-shaped) are well agreed upon. *Soft*, *fibrotic*, and *hard* are usually in disagreement. Compound markings (*FH*, *SF*, *SFH*, et cetera) are not plotted. They were even less frequently agreed to.

By inclusive definition, i.e., when every symbol of the compound marking is accepted just as if it stood alone, the number of pairings is much larger and the percentage of pairs in agreement is increased. Some of the increase is due to chance coincidences. The correction for chance is shown in figure 4.

By the *inclusive definition*, the number of comparisons was much larger. The agreement appeared much better, as would have been expected. *Soft*, which was often marked in combination with *hard*, emerged as the most reliable of the three, with 70 per cent of pairs in agreement. This was half again as good as the other two. The difference was real, not due to chance (99 per cent confidence level).

Under the *inclusive definition*, the likelihood of agreement just by chance is greatly enhanced. For an understanding of this, suppose the three symbols, *S*, *F*, and *H*, occurred with equal frequency. A lesion marked *S* by one would have a 33 per cent chance of being matched by another reader, if only single symbols were used. But if double symbols were admitted, then all such would have zero chance of agreeing with the single *S* reading by strict definition but 66 per cent chance of agreeing by inclusive definition. Taking into account the possible combinations and the observed frequencies of the several markings, the agreements

just by chance have been computed and are shown graphically in figure 4. If the remainder of the agreement is attributed to the readers perspicuity, then they showed up better by the strict definition (23 per cent compared to 16 per cent). But the level of reliability shown was much lower than the three radiologists had expected.

This was discouraging in view of the impression gained by the readers in a three-day conference that they were getting closer together. At a second three-day conference, the major effort was laid on studying together many films, especially those which had been diagrammed most diversely. The hope still was that all would learn "to speak the same language." It might be that the symbols

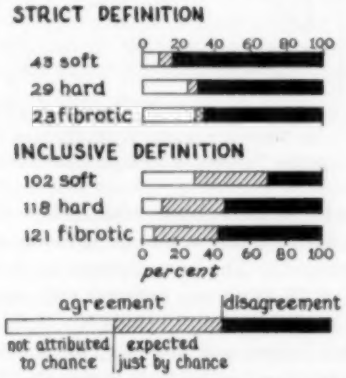


FIG. 4. Summary of agreement and disagreement on the principal categories, corrected for chance coincidences. The percentages are calculated on the basis of pairings. Each lesion marked *soft* would have afforded three pairs, except that those pairs were deleted when one reader marked the area *negative* or *nonpulmonary* or *nontuberculous*. The increase of chance coincidences for inclusive definition is due to the many overlappings of the compound markings, e.g., one marks the lesion *HS* and the other marks it *HF*. Of course, whenever one reader marks a lesion *SFH*, it practically ensures two "chance" agreements.

(*F*, *H*, *S*, et cetera) were not so "objective" and "non-polar" as had been hoped, for they gave no better agreement than the previous "active" and "inactive." Nevertheless, it was decided to give the code another trial, making some small revisions.

### *Code No. 2*

The *soft* category was divided into $S_1$, definite consolidation, and $S_2$, not quite so definite but still fuzzy and soft enough not to be placed in *H* category. *C* was introduced for *calcification* but, since it did not seem to be helpful, it is not mentioned further. The symbol *B* was introduced for *blebs* of emphysema. This addition was not particularly helpful at this time but, later, the concept was reintroduced under the name *honeycomb* in Series 3. The symbol *KR* was introduced to cover large hilar shadows judged not to be pathologic. The nontuberculous lesions were now to be marked *X* and described under remarks. For the

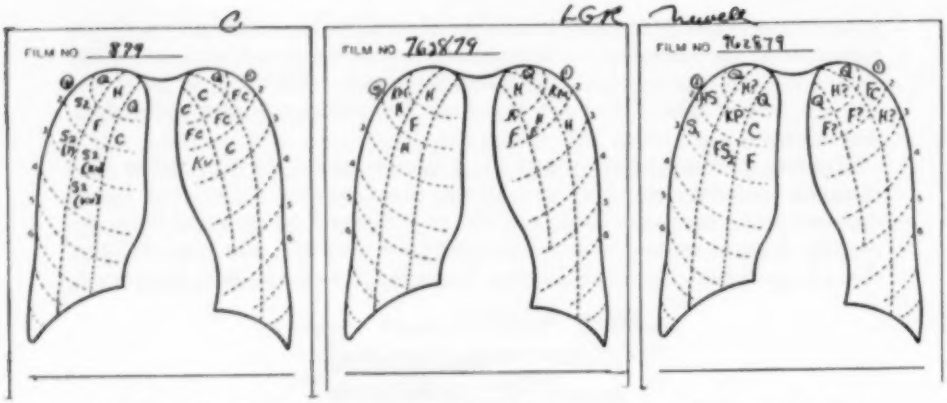


FIG. 5, Series 2. Illustrating the way the three readers marked the chest diagrams under Code No. 2. This example shows fewer compound markings than most of them and a larger than average number of "lozenges" occupied by significant shadows.

oblique lines used before to designate linear scars, a *Z* would now be used. It was found that the readers were making a mental division of each interspace into three parts (called "lozenges"). The new diagram is shown in figure 5.

*Series 2:* A fresh set of 50 films was selected and sent around to the three readers as before, to be read according to Code No. 2. The films were chosen to show rather less complex lesions and provided 138 areas designated as lesions by at least one reader (compared with 158 in Series 1). The agreement in regard to the presence of a (possibly) tuberculous lesion was much better than before (66 per cent triple agreement compared with 47 per cent triple agreement in Series 1). Reader W. E. C. still saw lesions as complex and reader R. R. N. marked them more simply, but the spread was reduced to one-half of that which occurred on the previous trial. This looked like improvement resulting from working together.

In regard to the designations *soft* and *fibrotic*, however, these two readers appeared to have overcompensated. In Series 1, reader W. E. C. recorded more *fibrotic* than *soft* and R. R. N. more *soft* than *fibrotic*. Obviously, both could not be right. But in Series 2, each had reversed his stand. This question of bias, as distinct from random error, is discussed later.

Reliability, on the whole, proved not to be any better than by Code No. 1. Improved agreement on *hard* and *fibrotic* was more than offset by the disagreement on *soft*. Even when $S_1$ and $S_2$ were lumped together, agreement was less than in Series 1. An attempt to find some lesions so surely *soft* that all could agree met with utter failure. Of 36 lesions marked *S* wholly or in part by at least one reader, there was only one triple agreement.

### *Code No. 3*

Readers W. E. C. and L. R. thereupon devised a "more objective" code. The diagram was similar to the one for Code No. 2, each interspace (anteriorly) being

divided into three parts (called "lozenges"). Below the diagram was printed these lists of categories:

| | | | |
|---|---|---|---|
| Homogeneous | Very dense | Band | Well-defined |
| Honeycombed | Moderately dense | Fanshaped | Moderately well-defined |
| Spotted | Not very dense | Linear | Poorly defined |
| Stippled | | Round | |
| Streaked | | Irregular | |

When the reader would see a lesion on the film which he considered possibly tuberculous, he would assign it an identifying number which he wrote in the corresponding "lozenge" on the diagram. He then entered this number after every class name which he thought was applicable to this lesion. If he thought the appearance was different in different parts of the lesion, he identified the parts by subscripts. In this way a complete descriptive classification was obtained for every portion of every lesion seen.

This code was used by readers W. E. C. and L. R. on 50 new films, selected for relative simplicity of disease. Reader R. R. N. read only 13 of this series. Of 92 lesions marked by one or the other, readers W. E. C. and LR each marked only 6 lesions which the other did not see. This was better agreement than on previous occasions. Nevertheless, in regard to description of the lesion, no progress was evident. Except in one category (*streaked*), disagreement was more frequent than agreement. For *stippled* and *linear* they did not agree in a single instance.

It was difficult for the investigators to accept the fact that descriptive words applied habitually by radiologists in their daily practice are as meaningless as the foregoing experience suggested. It was decided, therefore, to make one more attempt to obtain agreement, at least in the description of the texture.

*Series $S_2$:* New diagrams were printed, on which the "lozenges" were numbered (figure 6). There was only one list of categories:

**Smooth matrix**
**Spotted**
**Honeycombed**
**Checkered**
**Unclassified**

Each reader was to write after each of these class names the number of every "lozenge" in which he saw a lesion that fell into that category. The memorandum confirming the conference agreement as to the marking of this series is printed here verbatim:

*Code—Memorandum for Series $S_2$*

The purpose of the present division of our study is to find lesions that all three readers can all call by the same name, and we shall limit ourselves to the following classifications: "smooth matrix," "spotted," "honeycombed," "checkered," and "unclassified."

*"Smooth matrix":* The classification "smooth matrix" should be used every time the consultant is convinced that there is a homogeneous matrix on which any other detail is overlying or in which it is embedded. Whenever the consultant is convinced that the

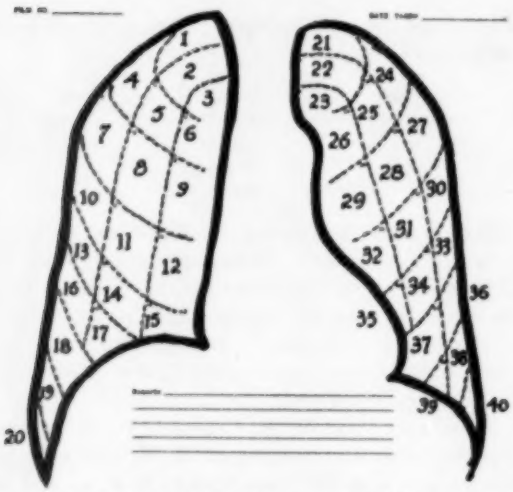


FIG. 6. Diagram used for Series $3_2$. Below this was printed, in type too small to show in the cut, this list of categories: *smooth matrix, spotted, honeycombed, checkered, unclassified.* After each of these the reader entered the numbers of every "lozenge" in which he saw a lesion to which that class name was applicable. Some categories might get one "lozenge" number, some several "lozenge" numbers, and some none at all on a given film.

other readers are likely to be impressed by the importance of the irregular detail, then to the classification "smooth matrix" he should add any other appropriate classification such as "plus honeycombed," or "plus spotted". By and large, "smooth matrix" means a predominantly smooth appearance with minor irregularities. Normal pulmonary vessels in front and behind the lesions are to be discounted.

*"Spotted":* Predominance (at least 75% of the lesion) of white spots with a dark reticulum.

*"Honeycombed":* Predominance (at least 75% of the lesion) of black spots with a white reticulum.

*"Checkered":* A mixture of "spotted" and "honeycombed" occupying about the central half of the continuum from "all spotted" to "all honeycombed." If a "spotted" lesion has more than 25% of its area "honeycombed" it should be classified as "checkered." The same procedure should be followed when more than 25% of a "honeycombed" lesion shows white spots. It is important that no one give a white spot of a given area greater weight in the differentiation of texture than a black spot of the same area. "Checkered" is a classification which allows the reader to deny that the lesion has a "smooth matrix" without specifying whether it is "spotted" or "honeycombed".

*"Unclassified":* All lesions where the consultant is in doubt as to the texture or where he is not certain that the other two consultants will classify the lesion in the same way.

A new set of 50 films was selected and sent around to the three readers as before. In a few weeks they were sent around again for a second reading.

Reader W. E. C. tended to use the largest number of descriptive terms (saw the lesions as complicated) and reader R. R. N. to use the fewest (saw them as simple). This was about as marked as in Series 2.

Reader L. R. saw only three-fifths as many *smooth-matrix* as either W. E. C.

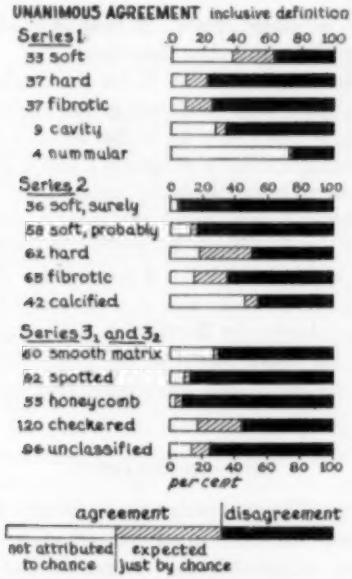


FIG. 7. Comparison of ability of three readers to agree on three different roentgenographic classifications of pulmonary lesions. The figures down the left margin show the numbers of lesions so classified by any reader. This plot is on the basis of unanimous agreement, in contrast to the other plots which are in pairs. Note that the only classes for which disagreement is not more frequent than agreement are *nummular* and *calcified*. *Soft* in Series 1 is offset by the two poor scores on *soft* in Series 2. *Hard* in Series 2 is not supported by *hard* in Series 1. Calculating standard errors on basis of the computed agreements by chance, and giving due weight to the fact that there are five classes of high frequency in Series 3 compared with three in Series 1, the difference in reliability between the best and the worst series appears insignificant.

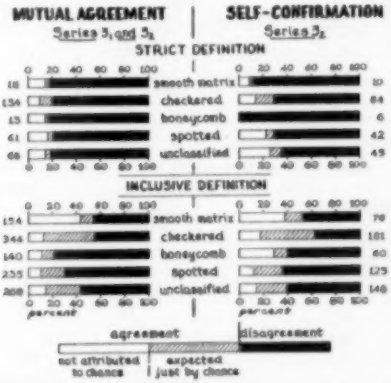


FIG. 8. Success in agreeing with another reader compared with success in repeating one's own first reading. The figures at the margin show the number of lesions so classified by any reader. The reliability for self-confirmation is just perceptibly better than for agreement with others.

or R. R. N., but twice as many *spotted*. A year of working together had not brought the readers any closer together than this!

Since the results of Series $3_1$ and Series $3_2$ for the categories common to both were quite similar, they have been combined in the analysis. It is to be remembered that reader R. R. N. read only 13 of the 50 films of Series $3_1$.

The results are set forth graphically in figures 7 and 8. It is evident that agreement on these terms was no better than for earlier Code No. 1. *Soft* of Series 1 and *smooth matrix* of Series 3 have much the same meaning; yet the later one won only half as many agreements as the earlier. As Series 1 presented choice among only 3 frequent classes and Series 3 presented 5 choices, the later scores might be said to contain more information in a ratio of 5 to 3. Correcting for this and allowing for one standard error fluctuation in the agreements attributed to chance, it is seen that the distributions in the two series did, in fact, overlap. It cannot be said with certainty that the readers had actually done less well in Series 3 than in Series 1.

### SELF-CONFIRMATION

Series $3_2$ was read twice. Figure 8 presents graphically the evidence for greater reliability in self-confirmation than in agreement with the other readers. The difference is small and, considering the computed amounts of agreement by chance, is barely significant.

TABLE 1

ESTIMATES OF ACTIVITY ON 100 SELECTED FILMS READ TWICE BY THREE READERS ACCORDING TO THE CODE OF THE NATIONAL TUBERCULOSIS ASSOCIATION

Series 0

| FIRST READING | SECOND READING | | | |
|---|---|---|---|---|
| | Active | ? Active | Inactive | Total |
| Active | 22 | 8 | 3 | 33 |
| Questionably active | 2 | 5 | 5 | 12 |
| Inactive | 4 | 6 | 42 | 52 |
| Total | 28 | 19 | 50 | 97 |
| ONE READER | ANOTHER READER | | | |
| | Active | ? Active | Inactive | Total |
| Active | 25 | 5 | 20 | 50 |
| Questionably active | 17 | 5 | 12 | 34 |
| Inactive | 17 | 10 | 52 | 79 |
| Total | 59 | 20 | 84 | 163 |

Each reading was compared with that of each other reader. Pairs were deleted if one reading was *negative* or *not pulmonary* or *nontuberculous*.

### IMPRESSION OF PROGNOSIS

For direct judgment of activity, readings on approximately 150 lesions were available.

Series 0, read twice, contained 100 films, with lesions on about half of them. The readers were instructed to mark them *active*, *probably active*, or *inactive*.

Series $3_1$, read once by two readers, 13 of them by all three readers, contained 50 films, practically all of which showed a lesion.

Series $3_2$, similar to $3_1$, was read twice by all three readers, who were instructed to mark every lesion *unstable* or *stable* if they believed such a judgment warranted. Unless they were sure in their own minds and believed that the other readers would agree, they were to mark the lesion *no judgment possible*. The readers understood that this prescribed attitude was different from their "natural" attitude on Series 0.

Statistical analysis shows no significant differences in reliability between readers. The data have therefore been combined in tables 1 and 2. An attempt has been made to present them graphically in figure 9. For *active* and *unstable*, pairings against other readers average 44 per cent in agreement. For *inactive* and *stable*, the average is 57 per cent in agreement. Contradictions make up most of the disagreements for Series 0. But for Series 3, contradictions are very few, *no judgment possible* making up almost the whole of the failures to agree.

When the reader's own first and second readings were paired, the self-confirmation on *active* and *unstable* was a third to a half better than the mutual

TABLE 2

DATA ON READERS' INCLINATION TO CALL LESIONS ACTIVE OR INACTIVE

ESTIMATE OF ACTIVITY

Series 3

| FIRST READING | SECOND READING | | | |
|---|---|---|---|---|
| | Unstable | No Judgment Possible | Stable | Total |
| Unstable | 26 | 18 | 1 | 45 |
| No judgment possible | 20 | 130 | 21 | 171 |
| Stable | 1 | 17 | 16 | 34 |
| Total | 47 | 165 | 38 | 250 |
| ONE READER | ANOTHER READER | | | |
| | Unstable | No Judgment Possible | Stable | Total |
| Unstable | 18 | 8 | 0 | 26 |
| No judgment possible | 41 | 99 | 18 | 158 |
| Stable | 2 | 16 | 14 | 32 |
| Total | 61 | 123 | 32 | 216 |

agreement. A similar result was obtained with respect to the designation *inactive*. No superiority was shown for *stable*, perhaps because of the large number of lesions marked *no judgment possible*. This suggests more willingness to make a definite diagnosis of instability than of stability, either because the uncoded criteria for activity were more clear in the reader's mind or because "conservatism" made him unwilling to promise a good prognosis, when he was uncertain.

Series 3 shows rather less reliability than Series 0. Urging the readers to be definite only when they felt sure did not increase the agreements. To be sure, it increased the over-all ratio of agreement to contradiction from 3 to 1 in Series

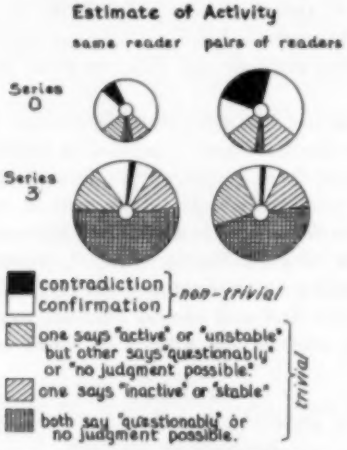


FIG. 9. Agreement between readers and self-confirmation on second reading by the same reader. In Series 0 the readers were permitted to mark a lesion *questionably active* if not willing to make a definite diagnosis of activity. In Series 3, they were instructed to write *no judgment possible* unless they were sure. Note that this results in a much greater reduction in the number marked *stable* (inactive) than in those marked *unstable* (active).

0 to 18 to 1 in Series 3. But this was at the cost of refusal to commit oneself 60 per cent of the time.

In Series 0, one-third of the readings were designated as *active*. In pairings one expects 11 per cent coincidence; the observed coincidence was 18 per cent of pairs in agreement. One-half of the readings were designated *inactive*, from which one would expect 25 per cent coincidence, and observed 36 per cent of pairs in agreement. Viewed in this way, approximately two-thirds of the agreement (including self-confirmation) might be accounted for by chance.

In Series 3, approximately one-third of the agreement might be accounted for by chance.

In Series 1, approximately one-fourth of the agreement on *soft*, *fibrotic*, *hard*, by the strict definition, appeared to be due to chance. The appearance of a larger area of agreement for the direct (intuitional) judgment of activity does not mean superiority as a diagnostic approach, but seemed to be due to the smaller number of classes (two significant categories).

TABLE 3

VARIABILITY IN THE BIAS OR SLANT OF THE THREE READERS, MEASURED BY RELATIVE NUMBERS OF ANTITHETICAL DIAGNOSES

| READER | SERIES | FIRST READING | | | SECOND READING | | | CHANGE IN SLANT |
|---|---|---|---|---|---|---|---|---|
| | | Cases Read | | Slant (Bias) | Cases Read | | Slant (Bias) | |
| | | Active | Inactive | | Active | Inactive | | |
| W. E. C. | 0 | 9 | 22 | −25° | 3 | 27 | −53° | −26° |
| R. R. N. | | 14 | 28 | −19° | 13 | 27 | −20° | −1° |
| L. R. | | 16 | 8 | +19° | 13 | 10 | +7° | −12° |
| | | Unstable | Stable | | Unstable | Stable | | |
| W. E. C. | 3 | 10 | 18 | −17° | 6 | 16 | −25° | −8° |
| R. R. N. | | 19 | 10 | +18° | 26 | 9 | +30° | 12° |
| L. R. | | 20 | 15 | +8° | 19 | 16 | +5° | −3° |
| | | Soft | Fibrotic | Slant (Bias) | Difference in Slant (Series 2 Minus Series 1) | | | |
| W. E. C. | 1 | 17 | 40 | −23° | +31° | | | |
| | 2 | 31 | 24 | +8° | | | | |
| R. R. N. | 1 | 48 | 22 | +21° | −44° | | | |
| | 2 | 12 | 28 | −23° | | | | |
| L. R. | 1 | 23 | 31 | −9° | −3° | | | |
| | 2 | 20 | 31 | −12° | | | | |

The transformation of ratio *active* to *inactive* into degrees of slant is illustrated in figure 10 and discussed in the text. In Series 0 and 3 the films were each read twice and the second set of readings is compared to the first. Series 1 and 2 were not read a second time. The two series are compared with each other. Note that W. E. C. finds a 31-degree difference in composition of the two series, while R. R. N. finds an even greater difference, but in the opposite direction. Analysis shows the personal bias not enough different, on the whole, between one reader and another, to be statistically significant in the presence of their great variability (*see* text). L. R. is, however, perceptibly less variable than W. E. C. or R. R. N.

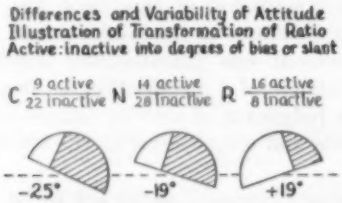
Differences and Variability of Attitude
Illustration of Transformation of Ratio
Active:Inactive into degrees of bias or slant
C 9 active / 22 inactive
N 14 active / 28 inactive
R 16 active / 8 inactive
-25°
-19°
+19°

FIG. 10. Illustrating the transformation of the ratio of lesions read *active* to lesions read *inactive* into degrees of slant or bias. Analytically, the slant is taken as the angle whose sine is $(A - I)/(A + I)$. This model fits in fair degree our intuitional judgment that a 10 per cent change from 90 per cent *A* to 100 per cent *A* requires an overwhelming slant toward *A*, whereas a very modest change in slant would shift a reader the 10 per cent from 45 per cent *A* to 55 per cent *A*.

### PERSONAL BIAS

The question arises how much of the disagreement was due to differences in bias between readers. Comment has already been made on reader W. E. C.'s inclination to see lesions as complex. If the "truth" for any series could be established, it would be possible to measure how far each reader inclined away from it. Not knowing the true number of active and inactive cases, it is possible only to compare each reader with the others. The data are presented in table 3. As the question is one of bias, slant, or inclination, it seemed natural to convert the ratios of *active* to *inactive* into degrees of slope. This is illustrated in figure 10.

In order to use the material from all of the series, it has to be assumed that the designations *active:inactive*, *unstable:stable*, and *soft:hard* are identical, or at least very similar semantic pairs. Proceeding on this assumption, analysis of variance failed to show any significant difference among the four series. Reader L. R. proved to be less than half as variable as R. R. N. (significant at 90 per cent level of confidence). No significant difference in bias (personal equation) could be shown among the three readers.

It was concluded that disagreements in judgment were mostly not on a basis of misunderstandings or personal differences in hopefulness toward tuberculosis. The better ability of a reader to confirm his own judgment on a film than to agree with another reader was not very great (figures 8 and 9). It becomes lost in the general unreliability when looked for by analysis of averages.

These readings were all on single chest films. Yerushalmy and co-workers (5) have since shown that readings of serial films for progression and regression of a visible lesion show fairly good self-confirmation and mutual agreement.

## Discussion

The present writers appear to have failed in this project of finding a reliable classification of the roentgenographic appearance or quality of a tuberculous pulmonary lesion. It is believed that reasonable degrees of tenacity and resourcefulness were used in the attempt. It was disappointing to find that many conferences and much practice, together and apart, failed to increase reliability and agreement to a useful degree.

Analysis shows that the amount of agreement was statistically significant, i.e., that differences in structure of the shadows were really being perceived. Nevertheless, the agreement was quantitatively insufficient for the purpose envisaged, namely, validation of a classification by long-time clinical studies.

The more subjective judgments of activity (intuitive) appeared to be about as reliable as description and classification of the objective appearance of the lesion on the film.

Success in classification (diagnosis) seems not to be attained by instructing the reader to "mark it only if you feel sure." To do this results either in an increase in disagreement or merely in an overwhelming reduction in the number of useful diagnoses ventured. The percentages of agreement and disagreement depend on how broadly agreement is defined. But the more generous one's defini-

tion of agreement, the less sharp the classification and the less use it will have for clinical purposes.

In discussing this research with others, radiologists and chest specialists, the writers find a general unwillingness to believe that descriptions of pulmonary lesions are as unreliable as were found in the present study. The present writers have become accustomed to this skepticism and believe that only those who have themselves made duplicate readings of a series of films can come to appreciate the hard fact of their own unreliability.

Even yet the conviction survives that the qualitative appearance of a lesion as seen on a chest film must have clinical significance. The present investigators believe that further effort should be made to find ways to bring out in optimal degree all of the information that may actually be there.


## Summary

Classification of possibly tuberculous pulmonary lesions on the basis of roentgenographic appearance is not very reliable. There is not much to choose among the three approaches to the problem: (*a*) an objective description of the lesion, (*b*) a quasi-pathologic classification, and (*c*) a subjective judgment of activity. All are less reliable than the classification of extent in common use (*minimal, moderately advanced, far advanced*).

To be sure, the last-named classification does not tell very much about pathology. None of the classifications approaches the reliability of roentgenographic detection of a pulmonary shadow, either in the hands of the present writers or as reported by others.

## Sumario

*Clasificación Descriptiva de las Sombras Pulmonares. Revelación de la Incertidumbre Reinante en el Diagnóstico Radiográfico de la Tuberculosis*

La clasificación de las lesiones pulmonares posiblemente tuberculosas a base del aspecto roentgenográfico no resulta muy fidedigna. No hay mucho que escoger entre las tres soluciones del problema: (*a*) una descripción objetiva de la lesión; (b) una clasificación cuasi-patológica; y (*c*) una apreciación subjetiva de la actividad. Las tres son menos dignas de confianza que la clasificación de la extensión en empleo hoy día (*mínima, moderadamente avanzada, muy avanzada*).

Por supuesto, esta última clasificación no nos indica mucho con respecto a anatomía patológica. Ninguna de las clasificaciones se compara en fidedignidad con el descubrimiento roentgenográfico de una sombra pulmonar, según han observado los AA. mismos o han comunicado otros.

## Resume

*Classification descriptive des ombres pulmonaires: Faillibilité du diagnostic de la tuberculose par la radiographie*

La classification des lésions pulmonaires pouvant être attribuées à la tuberculose sur la base de l'image radiographique n'est pas très sûre. Il n'y a guère à choisir entre les trois aspects suivants du problème: (*a*) La description objective de la lésion; (*b*) la classification semi-pathologique; (*c*) l'appréciation subjective de l'activité des lésions. Ces divers aspects sont moins sûrs que la classification usuelle selon l'extension de la tuberculose (*à minima, modérément avancée, très avancée*).

Il est toutefois évident que cette dernière classification ne donne que peu de renseignements sur les conditions pathologiques. D'après l'expérience des auteurs eux-mêmes, ou selon les communications d'autres auteurs, ces classifications sont encore moins sûres que la découverte d'une opacité pulmonaire.

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# COMPARISON BETWEEN THE INTERNATIONAL STANDARD TUBERCULINS, PPD-S AND OLD TUBERCULIN[1, 2]

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## INTRODUCTION

Since purified protein derivative tuberculin, PPD-S (1), has been chosen to serve as the international standard for human (PPD) tuberculins (2), it is important to know precisely how it compares with the lot of OT which has been serving as the international standard for Old Tuberculins. The following study was made in order to compare the two preparations and to assess any differences between them. The study will also show the differences which exist in basically different tuberculins.

## MATERIALS AND METHODS

PPD-S for testing was made from the stock lyophilized standard product (1) into a 1 per cent solution. The diluent used had been prepared by dissolving 4.774 gm. of $Na_2HPO_4 \cdot 12H_2O$, 0.363 gm. of $KH_2PO_4$, and 8 gm. of NaCl in one liter of freshly distilled water and sterilizing. Dilutions of this were then made to give 0.00002 and 0.005 mg. per 0.1 ml.

PPD made in the Weybridge Laboratories, England, by trichloracetic acid precipitation (3), and supplied as a 0.2 per cent solution by Drs. H. H. Green and A. B. Paterson was also tested. Dilutions of this were prepared in the same way to give 0.00002 and 0.005 mg. doses for comparison with the corresponding doses of PPD-S.

The OT (international standard) was kindly supplied by Dr. K. Jerne from the Statens Serum Institute, Copenhagen. Dilutions of 1:10,000 or 1:5,000, with the same buffer as given above, were tested against the usual first dose (0.00002 mg.) of PPD-S. Those patients who did not give a reaction of 5 mm. or more to this dose in forty-eight hours were tested with 1:100 or 1:80 of OT, and 0.005 mg. of PPD-S.

One-tenth of a milliliter of the proper dilutions of the tuberculins was injected intradermally into the volar surface of each of the two forearms approximately 2 inches below the elbow. After forty-eight hours the amount of induration of the reactions was measured as two diameters at right angles to each other, and the height of the reaction was estimated on a scale of 1, 2, 3, and 4. The height and the average of the two diameters were recorded (*see* table 1). On alternate patients the arms were reversed and the identity of the product used in each site was not known to the observer when the readings were made. Only two observers were engaged in these studies, and they were known to read reactions very closely for many years. The results were evaluated statistically by Mr. W. J. Carnahan of the Philadelphia Tuberculosis and Health Association, to whom the writers express their appreciation.

The patients tested were those who attended the clinics of the Henry Phipps Institute for family check-up and diagnosis.

## RESULTS

The comparative results for the two tuberculins are given in table 1, and the statements made below were found to be statistically valid. They show that, in

[1] From the Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pennsylvania.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

TABLE 1

COMPARATIVE SKIN REACTIONS WITH INTERNATIONAL STANDARD TUBERCULINS, OT AND PPD-S

| SUBSTANCE TESTED | DOSE IN 0.1 ML. | NUMBER OF PATIENTS TESTED | NUMBER OF PATIENTS POSITIVE | MEAN SIZE OF REACTIONS* IN MM. | DIFFERENCE IN MEAN SIZE (PPD-S MINUS OT) | STANDARD ERROR OF DIFFERENCE IN DIAMETERS | $t$ | PROBABILITY |
|---|---|---|---|---|---|---|---|---|
| PPD-S | 0.00002 mg. | 114 | 62 | 26.9 × 2.6 | +4.3 × 0.1 | 0.56 | 7.75 | P <0.001 |
| OT (I.S.) | 1:10,000 = "0.01 mg." | 114 | 61 | 22.6 × 2.5 | | | | |
| PPD-S | 0.00002 mg. | 87 | 50 | 25.5 × 2.5 | +5.6 × 0.2 | 0.48 | 11.71 | P <0.001 |
| OT (I.S.) | 1:10,000 | 87 | 49 | 19.9 × 2.3 | | | | |
| PPD-S | 0.00002 mg. | 83 | 70 | 28.2 × 2.5 | +0.3 × 0.1 | 0.59 | 0.53 | P >0.50 |
| OT (I.S.) | 1:5,000 | 83 | 71 | 27.9 × 2.4 | | | | |
| PPD-S | 0.005 mg. (250×) | 41 | 21 | 23.1 × 2.4 | +4.4 × 0.2 | 0.89 | 4.99 | P <0.001 |
| OT (I.S.) | 1:100 (50×) | 41 | 16 | 18.7 × 2.2 | | | | |
| PPD-S | 0.005 mg. | 14 | 14 | 22.1 × 1.8 | +2.8 × 0.1 | 2.16 | 0.84 | P >0.40 |
| OT (I.S.) | 1:80 (62.5×) | 14 | 12 | 19.3 × 1.7 | | | | |
| PPD-S | 0.005 mg. | 27 | 16 | 20.3 × 1.9 | −5.5 × 0.2 | 1.14 | 4.82 | P <0.001 |
| OT (I.S.) | 1:40 (125×) | 27 | 15 | 25.8 × 2.1 | | | | |

* The mean diameters were based on only the reactions which were positive with both tuberculins.

I.S. = international standard.

the case of those persons who were sufficiently sensitive to react to 0.00002 mg. of PPD-S (or 1:50,000 of a solution of which 1 ml. = 10 mg.), the reaction to the international standard OT approximately equaled that with the PPD-S only when the OT was diluted 1:5,000. From this comparison it would be assumed that the OT solution corresponds in strength to a PPD-S solution of which 1 ml. = 1 mg., or that it is one-tenth as strong as the 1 per cent PPD-S stock solution used as standard. However, on the less sensitive patients, or those who reacted only to the second dose, 0.005 mg. of PPD-S (an increment in dosage of 250 times), or 1:200 of the stock solution, the OT had to be diluted only to 1:80, or an increment of 62.5 times, in order to give equivalent reactions. In this case it would appear that the OT solution corresponds in strength to a PPD-S solution of which 1 ml. = 4 mg. or that it is two-fifths as strong as the PPD-S stock solution. Therefore, at this dosage level the OT appears to be four times stronger than it did at the low dosage level. These relationships are more clearly portrayed as follows.

If the strengths of the stock solutions be calculated on the basis of equivalence in potency at the first dose level of sensitivity, as shown below, then at the second dose level the OT is four times as potent as the PPD-S, since it required one-fourth the amount to give equal reactions.

First dose level, equivalent potencies—
0.1 cc. 1:50,000 PPD-S = 0.00002 mg.
(stock solution—1 cc. = 10 mg. PPD-S)
0.1 cc. 1: 5,000 OT(international standard) = 0.00002 mg.
(stock solution—1 cc. = 1 mg. PPD-S)
Second dose level, equivalent potencies—
0.1 cc. 1:200 PPD-S = 0.005 mg.
0.1 cc. 1: 80 OT(international standard) = 0.00125 mg.

On the contrary, if the strength of the stock solutions be calculated on the basis of equivalence in potency at the second dose level of sensitivity, as shown below, then at the first dose level the OT has only one-fourth the potency of PPD-S, since it required four times as much to give equal reactions.

Second dose level, equivalent potencies—
0.1 cc. 1:200 PPD-S = 0.005 mg.
(stock solution—1 cc. = 10 mg. PPD-S)
0.1 cc. 1: 80 OT(international standard) = 0.005 mg.
(stock solution—1 cc. = 4 mg. PPD-S)
First dose level, equivalent potencies—
0.1 cc. 1:50,000 PPD-S = 0.00002 mg.
0.1 cc. 1: 5,000 OT(international standard) = 0.00008 mg.

These results indicate that different antigens in these two tuberculins are exerting their effects in people with different sensitivities. This hypothesis is supported by the fact that reactions are frequently missed altogether with one of the test substances (*see* table 1), and this almost never occurs when the same or similar tuberculins are compared.

In table 2 it is shown that approximately equal reactions were obtained, regardless of which arm was used, when the same dose of the same tuberculin, PPD-S, was applied to both arms. Moreover, a 20 per cent increase in the dose of PPD-S was not noticeable, but a 50 per cent increase in dosage just began to show a significant increment in the size of the reaction.

It is also shown in table 2 that the PPD made in the Weybridge Laboratories was similar in potency to the standard PPD-S at both low and high dosage levels, indicating that they are qualitatively similar.

## DISCUSSION

Tuberculin solutions can be made of any desired strength by arbitrarily diluting weighed amounts of a product, such as PPD-S. A 1 per cent solution (1 ml. = 10 mg.) was chosen as a stock solution of suitable strength. A dose of 0.00002 mg. of PPD-S was chosen (4) as a suitable first dose because it rarely gave a 4-plus reaction and yet detected 96 per cent of clinically significant cases of tuberculosis (5).

With Old Tuberculins, arbitrary dilutions can also be made, but it is very difficult to know what the concentration of the active principle is in the original Old Tuberculin. It was shown (7) that the amount of the active protein or proteins present in any tubercle bacillus culture filtrate and, therefore, in any Old Tuberculin made from it depends chiefly upon the strain of tubercle bacillus used, upon the time of growth, and possibly upon many other factors, such as culture media, pH, and temperature. Some Old Tuberculins, therefore, are considerably more potent than others. The amounts of active protein which would be contained in the 0.1 ml. doses of different dilutions of OT, depending upon the strength of the original OT, are shown in table 3.

The commonly used terminology for OT (1 ml. = 1,000 "mg.") has been based upon the false assumption that 1 ml. weighs 1,000 mg. These "milligrams," therefore, are not to be confused with real milligrams of active material. Thus, for each of the doses, 1.0, 0.1, and 0.01 "mg." (column 2, table 3), the corresponding real milligrams are given in columns 3, 4, and 5, according to the strength of the original stock OT.

The data in table 3 show, for example, that the dose given in a 1:10,000 dilution of a strong OT, such as one having 10 mg. of active protein per ml., is 0.0001 mg., whereas in a weak OT (2 mg. per ml.) the dose would be 0.00002 mg. Comparisons frequently recorded in the literature (6), in which a dose of 0.0001 mg. PPD-S was appraised against 0.1 "mg." of a strong OT, whose equivalence was actually 0.001 mg. of PPD-S, are only confusing and valueless.

This kind of mathematical equivalence of dosages holds true at any level of sensitivity of the patients in the comparison of two tuberculins which are qualitatively the same. Thus, since the PPD made in the Weybridge Laboratories matched the PPD-S at high and low dosage levels, it is suggestive that it contains approximately similar proportions of the active proteins. On the other hand, the equivalence did not hold true in the comparisons between PPD-S and Old Tuberculin (international standard). The differences found in the relative potencies of

TABLE 2

VARIATIONS IN THE REACTIONS TO THE SAME AND DIFFERENT TUBERCULINS

| SUBSTANCE TESTED | ARM USED | DOSE (MG.) IN 0.1 ML. | NUMBER OF PATIENTS TESTED | NUMBER OF PATIENTS POSITIVE | MEAN SIZE OF REACTIONS IN MM.[1] | DIFFERENCE IN MEAN SIZE OF REACTIONS | STANDARD ERROR OF DIFFERENCE IN DIAMETER | $t$ | PROBABILITY |
|---|---|---|---|---|---|---|---|---|---|
| PPD-S | Left | 0.00002 | 41 | 26 | 29.4 × 2.6 | 1.2 × 0 | 0.59 | 2.08 | P >0.04 |
| PPD-S | Right | 0.00002 | 41 | 26 | 30.6 × 2.6 | | | | |
| PPD-S | Right | 0.000024 | 57 | 22 | 32.7 × 2.8 | | | | |
| PPD-S | Left | 0.00002 | 57 | 22 | 32.6 × 2.7 | 0.1 × 0.1 | 0.42 | 0.17 | P >0.90 |
| PPD-S | Left | 0.000024 | 47 | 33 | 32.1 × 2.7 | | | | |
| PPD-S | Right | 0.00002 | 47 | 33 | 32.3 × 2.7 | 0.2 × 0 | 0.31 | 0.65 | P >0.50 |
| PPD-S | Left | 0.00002 | 26 | 17 | 26.7 × 2.4 | | | | |
| PPD-S | Right | 0.00003 | 26 | 17 | 29.7 × 2.5 | 3.0 × 0.1 | 1.17 | 2.59 | P >0.02 |
| PPD-S | Right | 0.00002 | 18 | 10 | 34.0 × 2.9 | | | | |
| PPD-S | Left | 0.00003 | 18 | 10 | 37.9 × 3.0 | 3.9 × 0 | 1.32 | 2.95 | P >0.01 |
| PPD-S | Right | 0.006 | 33 | 21 | 22.9 × 2.1 | | | | |
| PPD-S | Left | 0.005 | 33 | 21 | 22.2 × 2.1 | 0.7 × 0 | 0.52 | 1.37 | P >0.20 |
| PPD-S | Left | 0.00002 | 66 | 38 | 27.6 × 2.5 | | | | |
| PPD-Weybridge | Right | 0.00002 | 66 | 38 | 27.3 × 2.5 | 0.4 × 0 | 0.34 | 1.00 | P >0.30 |
| PPD-S | Left | 0.005 | 23 | 14 | 18.9 × 2.2 | | | | |
| PPD-Weybridge | Right | 0.005 | 23 | 14 | 19.2 × 2.1 | 0.3 × 0.1 | 1.72 | 0.15 | P >0.80 |

TABLE 3

DOSAGES OF OLD TUBERCULIN OF DIFFERENT STRENGTHS

| DILUTION | USUAL TERMINOLOGY IN THE LITERATURE | IF THE ORIGINAL OT STOCK SOLUTION CONTAINED AN EQUIVALENT OF PURE PROTEIN EQUAL TO: | | |
|---|---|---|---|---|
| | | 10 mg. per ml. | 5 mg. per ml. | 2 mg. per ml. |
| Stock | 1,000 "mg." per ml. | | | |
| 1:100 | 1.0 cc. = 10.0 "mg." | 1 cc. = 0.1 mg. | 1 cc. = 0.05 mg. | 1 cc. = 0.02 mg. |
| | 0.1 cc. = *1.0* "mg." | 0.1 cc. = 0.01 mg. | 0.1 cc. = *0.005* mg. | 0.1 cc. = 0.002 mg. |
| 1:1,000 | 1.0 cc. = 1.0 "mg." | 1 cc. = 0.01 mg. | 1 cc. = 0.005 mg. | 1 cc. = 0.002 mg. |
| | 0.1 cc. = *0.1* "mg." | 0.1 cc. = 0.001 mg. | 0.1 cc. = 0.0005 mg. | 0.1 cc. = 0.0002 mg. |
| 1:10,000 | 1.0 cc. = 0.1 "mg." | 1 cc. = 0.001 mg. | 1 cc. = 0.0005 mg. | 1 cc. = 0.0002 mg. |
| | 0.1 cc. = *0.01* "mg." | 0.1 cc. = *0.0001* mg. | 0.1 cc. = 0.00005 mg. | 0.1 cc. = *0.00002* mg. |

The italicized doses are the ones commonly used in practice.

these two tuberculins at different dosage levels indicate that they are qualitatively different. The method advocated by A. A. Miles (8) of comparing these same two standard tuberculins also shows a dissimilarity between them. In fact, this difference between an OT and PPD (9) was pointed out by Seideman (10) as far back as 1939. Such a qualitative difference can probably be explained on the basis that several antigens are present in both PPD and OT and that their percentage concentrations vary in the two tuberculins. Moreover, Old Tuberculins contain also carbohydrate and nucleic acid, and, in addition, nonspecific substances which were actually isolated (11) from one commercial OT. These substances gave nonspecific reactions in the larger doses and would undoubtedly enhance the specific reactions to these doses.

It is evident from previous chemical analyses and electrophoresis studies that PPD-S does contain more than one tuberculin protein. It is possible that one of the proteins may be much more specific than the others, and another one may be responsible for a cross-specificity. For a more extended discussion see the review article (12).

Proteins, demonstrated to be different in their chemical analyses (13) and physicochemical properties, have been isolated from tuberculin (7), and it was shown by Seibert, Crumb, and DuFour (14) that they had different immunologic specificities. Moreover, these different proteins exhibited qualitative differences in potency when used for skin testing. Some were more potent than others when tested in equivalent doses based on their nitrogen contents. This was discussed in more detail in articles previously published (12, 15). Even more potent fractions than those so far reported have been isolated and are being studied. The establishment of the purity, stability, and reproducibility of such products, however, needs considerable investigation before they can be recommended for practical use.

Nissen Meyer (16) stated that this type of "asymmetrical correlation of responses to two preparations may suggest that the population under test has been sensitized by different antigens." Conversely, among the antigens which have been demonstrated to give an asymmetrical correlation of responses, it is reasonable to hope that the desired test product for each type of sensitization may be found.

It was pointed out by Nissen Meyer (16) that the purified tuberculin, RT, made in the Copenhagen laboratories differed only slightly in a qualitative sense from the PPD-S, a difference which could be detected only on the less sensitive patients. This confirms the present results with these two tuberculins. This fact, as well as the fact that different preparations made in their laboratory have different unit values, may be explained on the basis of the presence of different relative amounts of the various proteins of different specificities. Moreover, some of the antigens may be diluted out of the potency range in the higher dilutions, as was also done with the nonspecific substances in OT (11). If this is so, then obviously the elimination of nonspecific substances and the attempt to isolate the individual proteins in reproducibly pure form are strongly indicated. Moreover, the large-scale testing with these purified antigens on populations known to

have different types of sensitivity, such as have been found by Palmer and his collaborators (17, 18), may demonstrate desired specificities for the different antigens.


## SUMMARY

Intradermal reactions to the international standard tuberculins, Old Tuberculin (OT) and purified protein derivative (PPD-S), were compared in clinic patients and found to give reactions of different intensities at different dosage levels.

When the dilution of the OT (1:5,000) was found at which it gave equivalent reactions to 0.00002 mg. of PPD-S in highly sensitive patients, it was noted that the dilutions at which the two tuberculins were equivalent in the less sensitive patients were not in the same proportion. Instead, the OT appeared to be four times as potent as the PPD-S at the lower dosage level (1:80 versus 0.005 mg.).

Conversely, if 0.1 ml. of the dilution of OT (1:80) was considered to be equivalent to 0.005 mg. of PPD-S on the basis of equivalent reactions on less sensitive patients, then the PPD-S appeared to be four times stronger than the OT when tested on the more sensitive patients.

These results suggest that the two tuberculins are qualitatively different. In addition to the presence of nonspecific substances in the OT, it is possible that the two tuberculins have different antigens with different specificities, but they may also have different proportions of some of the same antigens.

The PPD made in the Weybridge laboratories was found to give reactions of similar intensity to those given by PPD-S at both dosage levels.

## SUMARIO

*Comparación entre las Tuberculinas de Tipo Internacional, PPD-S y la Tuberculina Antigua*

Al comparar en enfermos de clínica las intradermorreacciones a las Tuberculinas de Tipo Internacional, la Tuberculina Antigua y al PPD-S, observóse que las mismas alcanzaban distinta intensidad con dosis de diverso tamaño.

Cuando se descubrió la dilución (1:5,000) a la que la Tuberculina Antigua producía reacciones equivalentes a las de 0.00002 de PPD-S en enfermos muy sensibles, se notó que no guardaban la misma proporción las diluciones a las que las dos tuberculinas eran equivalentes en los enfermos menos sensibles. En vez de ello, la Tuberculina Antigua parecía ser cuatro veces más potente que el PPD-S a las dosis más bajas (1:80 comparado con 0.005 mg.)

En cambio, si se consideraba que 0.1 ml. de la dilución de Tuberculina Antigua (1:80) era equivalente a 0.005 mg. de PPD-S a base de las reacciones equivalentes en los enfermos menos sensibles, el PPD-S parecía ser cuatro veces más potente que la Tuberculina Antigua al hacerse la comprobación en los enfermos más sensibles.

Sugieren esos resultados que las dos tuberculinas son cualitativamente distintas. Además de la presencia de substancias anespecíficas en la Tuberculina Antigua, es posible que las dos tuberculinas posean distintos antígenos dotados de distintas especificidades, pero pueden también contener distintas proporciones de los mismos antígenos.

El PPD elaborado en los laboratorios de Weybridge produjo, según se observó, reacciones de intensidad semejante a las producidas por el PPD-S con ambas clases de dosis.

## RESUME

*Comparaison entre les tuberculines standard international, PPD-S et tuberculine de Koch (Old Tuberculin)*

Les réactions intradermiques aux tuberculines étalons, PPD-S et vieille tuberculine, furent comparées chez des malades de clinique et on a constaté qu'aux différentes concentrations elles produisaient des réactions d'intensité différente.

Lorsqu'il a été démontré que la dilution de la tuberculine de Koch (1:5.000) produisait des réactions équivalentes à celles de 0,00002 mg. de PPD-S chez des sujets à sensibilité très élevée, on a observé également que les dilutions auxquelles les deux tuberculines se montraient équivalentes chez des sujets moins sensibles n'étaient pas au même titre. Au lieu de cela, la tuberculine de Koch (old tuberculin) s'est montrée quatre fois plus active que la tuberculine PPD-S au titre plus faible (1:80 contre 0,005 mg.).

Au contraire, si 0,1 ml. de la dilution de tuberculine de Koch (1:80) était considéré l'équivalent de 0,005 mg. de PPD-S sur la base de réactions équivalentes chez des sujets moins sensibles, la PPD-S se montrait alors quatre fois plus forte que la tuberculine de Koch quand elle était soumise au test chez les sujets plus sensibles.

Ces résultats suggèrent qu'il existe une différence qualitative entre les deux tuberculines. Outre la présence de substances non spécifiques dans la tuberculine de Koch (old tuberculin), il est possible que ces deux tuberculines renferment des antigènes dont la spécificité diffère, comme elles peuvent aussi avoir des proportions différentes des mêmes antigènes.

La PPD préparée dans les laboratoires de Weybridge a donné des réactions de même intensité que celles produites par la PPD-S aux deux concentrations.


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# COMPARISON OF PLASMA VISCOSITY AND ERYTHROCYTE SEDIMENTATION RATES IN PULMONARY TUBERCULOSIS[1,2]

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## INTRODUCTION

The present study is a continuation of previous work which concerned itself with the plasma viscosity values for apparently healthy adults and persons ill with known disease (1). It was concluded that plasma viscosity offers valuable evidence in clinical medicine for the determination of organic disease which may cause physiochemical plasma alterations. The comparative results of plasma viscosity and erythrocyte sedimentation rate determinations in patients ill with pulmonary tuberculosis are summarized in the present report.

## MATERIALS AND METHODS

The plasma viscosity was measured by estimating the resistance to flow of plasma compared with that of distilled water under similar conditions in regard to velocity of flow, length and diameter of capillary tube, and particular temperature (20°C.) at the time of determination.

A mixture of 2 per cent potassium oxalate and 3 per cent ammonium oxalate was used as the anticoagulant. All the blood specimens were withdrawn during the morning. The sedimentation rates were determined the same morning. The tubes were then centrifuged. The plasma and distilled water tubes were placed in a water bath at 20°C. The plasma and distilled water were removed from the water bath and the viscosities were immediately compared.

Three readings were made on each specimen. The tubes were then emptied by suction and cleaned with distilled water, ether alcohol mixture, and dried with air. Then, the next specimens of plasma and distilled water were removed from the water bath and compared.

Using the above anticoagulant, the viscosities on any single specimen appeared to be identical when measured up to three days later. It was apparent after many experimental attempts that the readings were identical when rapid suction by mouth was used.

The viscosity tubes were calibrated to read to 0.1. A variation of 1°C. in the temperature of the water bath did not seem to change the final reading. Despite the fact that there is a variation from the mean under basal conditions of exercise, rest, and diet, when the viscosity was measured to the first decimal place, no significant change was found. A change of plus or minus 1 per cent will be found under such conditions if the viscosity is measured to the second decimal place (2). This was found to be the case with the Whittington type of viscometer, which has an accuracy of approximately 0.2 per cent. The latter instrument is not adapted for large-scale routine work. The simple apparatus used in the present study could give 18 to 20 determinations in an hour.

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[1] From Vermont Sanatorium, Pittsford, Vermont.

[2] Presented, in part, before the Medical Session, as part of the session on *Research*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, on May 21, 1953.

TABLE 1

COMPARISON OF SERIAL STUDIES OF PLASMA VISCOSITY AND ERYTHROCYTE SEDIMENTATION RATES OF 420 PATIENTS WITH PULMONARY TUBERCULOSIS

| STATUS OF DISEASE | NORMAL FINDINGS<br>Erythrocyte sedimentation rate = 12 mm. and less per hour.<br>Viscosity = 1.5 to 1.7 × $H_2O$ at 20°C. | ABNORMAL FINDINGS<br>Erythrocyte sedimentation rate = more than 12 mm. per hour.<br>Viscosity = 1.8 to 2.8 × $H_2O$ at 20°C. |
|---|---|---|
| Active | Erythrocyte sedimentation rate = 15%<br>Viscosity = 5% | Erythrocyte sedimentation rate = 85%<br>Viscosity = 95% |
| Inactive | Erythrocyte sedimentation rate = 80%<br>Viscosity = 98% | Erythrocyte sedimentation rate = 20%<br>Viscosity = 2% |

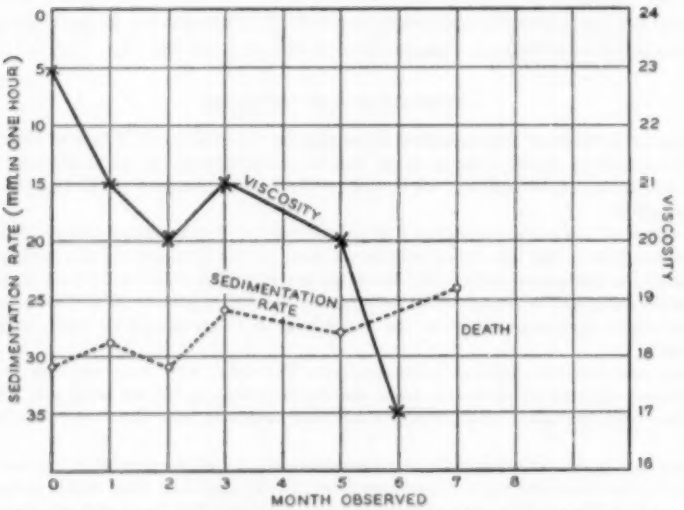


FIG. 1. Far advanced pulmonary tuberculosis: male, sixty-six years old

## OBSERVATIONS

The study was concerned with 420 patients ill with pulmonary tuberculosis who were followed at monthly intervals.[1] More than 5,000 determinations were made during a period of four years.

As may be seen in table 1, values for the erythrocyte sedimentation rates were normal in 15 per cent of the patients who had active pulmonary tuberculosis; whereas, in the same group, the plasma viscosity was consistently normal in 5

[1] Clinical classification: *active*—sputum and gastric contents almost always contained tubercle bacilli, lesions unstable by roentgenographic examination; *inactive*—sputum and gastric contents negative for tubercle bacilli by culture, lesions stable by roentgenographic examination. All conditions existed for at least three months.

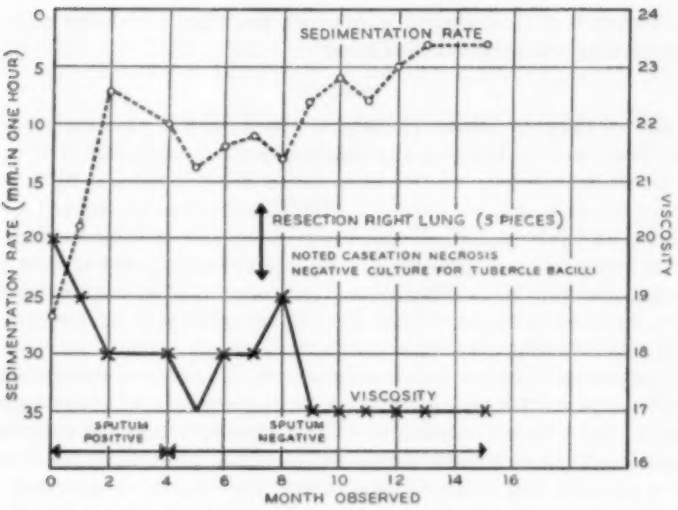


FIG. 2. Moderately advanced pulmonary tuberculosis: female, twenty-seven years old

per cent. In other words, abnormal plasma viscosity values were consistently present in 95 per cent of the active cases of pulmonary tuberculosis, and abnormal erythrocyte sedimentation rates were present in 85 per cent of this same group of patients. Normal erythrocyte sedimentation values were repeatedly present in 80 per cent of the patients with inactive lesions, while the plasma viscosity values were repeatedly within normal limits in 98 per cent of the same group.

There was no relation between the plasma viscosity and the hematocrit values. Anemia did not change the plasma viscosity. There was no relation between the plasma viscosity and menstruation. There was a small rise in viscosity during the last three months of pregnancy of no more than 0.1, so that patients with inactive pulmonary tuberculosis would frequently have a plasma viscosity of 1.8. This would persist for approximately one to two months following delivery. There was no relation between total protein and plasma viscosity. Patients who appeared acutely and fatally ill would very often experience a sudden drop in plasma viscosity value to a normal level of 1.7. When this occurred, death followed within approximately three months (figure 1).

The highest plasma viscosity value found was 2.8, and the lowest was 1.5. The plasma viscosity in inactive tuberculosis consistently remained at 1.7. Those patients who showed a plasma viscosity of 1.8 or more revealed evidence of active pulmonary tuberculosis or, if inactive, some other inflammatory, hyperplastic, or degenerative process. The exception was the pregnant patient, as stated previously.

Figure 2 is an example of a case in which apparently complete removal of tuberculous tissue resulted in the return of the plasma viscosity values to normal within two months. Patients with frank caseation tuberculosis appeared to show

closer correlation of their clinical status with the plasma viscosity values than with the erythrocyte sedimentation rates.

## COMMENT

The normal range of plasma viscosity is well defined in most instances under constant temperature. Utilizing the simple apparatus employed in the present study, the plasma viscosity was 1.7 times that of distilled water at 20°C. It rarely falls below this value but can reach 1.5. When the plasma viscosity is 1.8 or more and persists at this level, some disease process consisting of inflammation, hyperplasia, or degeneration is present. The plasma viscosity is not specific for any disease process, but when other disease processes can be eliminated, plasma viscosity increases with the clinical severity of pulmonary tuberculosis. With recovery, the viscosity will return to its normal range.

The plasma viscosity is a better reflection of the status of tuberculosis than the erythrocyte sedimentation rate. It is more sensitive and is not apparently changed by slight variations in regular living, season, sex, age, or menstruation.

The plasma viscosity can be estimated by several techniques. Some methods are more accurate and sensitive than others. The apparatus described can be used in clinical practice when the values are compared following serial determinations.

## CONCLUSIONS

A study is presented of the comparison of plasma viscosity determinations and erythrocyte sedimentation rates obtained periodically in 420 patients with pulmonary tuberculosis who were followed at monthly intervals. The conclusion was reached that the value for plasma viscosity is a more sensitive indicator of the presence and degree of activity of pulmonary tuberculosis than the erythrocyte sedimentation rate.

## CONCLUSIONES

*Comparación de la Viscosidad del Plasma y de los Coeficientes de Eritrosedimentación en la Tuberculosis Pulmonar*

En este estudio, compáranse en serie la viscosidad del plasma y los coeficientes de eritrosedimentación en 420 tuberculosos pulmonares observados mensualmente.

La conclusión alcanzada es de que la prueba de la viscosidad del plasma constituye un indicador más sensible de la presencia y grado de actividad de la tuberculosis pulmonar que el coeficiente de sedimentación.

## CONCLUSIONS

*Comparaison entre la viscosité du plasma et la vitesse de sédimentation dans la tuberculose pulmonaire*

Présentation d'une étude comparative de la viscosité plasmatique et de la vitesse de sédimentation dans une série de 420 cas de tuberculose pulmonaire examinés mensuellement.

En conclusion, le test de viscosité plasmatique est un indicateur plus sensible de la présence et du degré d'activité de la tuberculose pulmonaire que la vitesse de sédimentation.

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# EVALUATION OF THE OXIDATION-REDUCTION DYE TEST FOR THE DETERMINATION OF VIRULENCE OF MYCOBACTERIA IN VITRO[1]

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## INTRODUCTION

The determination of virulence of species of *Mycobacterium* by a rapid *in vitro* method would be of particular value in the laboratory diagnosis of tuberculosis. A spot-plate test utilizing certain oxidation-reduction dyes was developed in this laboratory by Wilson, Kalish, and Fish (1) in 1952, and, in a preliminary study of 271 acid-fast cultures, there was complete agreement between the results obtained in the dye reaction and those obtained in animal pathogenicity studies. The substitution of a tube test for the more hazardous plate test procedure was reported by Wrinkle and Patnode (2). For obtaining a more thorough evaluation of the oxidation-reduction test, an additional 760 cultures of *Mycobacterium* were examined.

## MATERIALS AND METHODS

The dye test was evaluated on 572 cultures of *Mycobacterium* isolated in this laboratory from pathologic specimens of sputum, gastric washings, body fluids, and bronchial washings, and on 188 strains of acid-fast organisms sent to this laboratory as pure cultures for identification.

The procedures employed for the test were essentially those described by Wilson and associates (1), except that five drops of each of the four dyes were placed in four cotton-stoppered test tubes (10 x 75 mm.) by means of capillary pipettes (2). The four dyes employed were phenol indophenol, sodium benzenoneindophenol, sodium benzenoneindo-3′-chlorophenol, and sodium 2,6-dibromobenzenone-indophenol. Approximately 5 mg. of each of the cultures were added to each of the four tubes of dye. The tubes were placed in a test-tube rack, shaken, and examined against a white background at the end of fifteen minutes. Each series of tests was controlled by simultaneous tests with an avirulent acid-fast saprophyte, *Mycobacterium phlei*, and a virulent strain of *Mycobacterium tuberculosis* var. *hominis*, H37Rv. A satisfactory test was indicated when the four dyes containing *M. phlei* were decolorized completely, while those containing the H37Rv strain retained their color. In some instances it was necessary to dilute the dyes further in order to obtain satisfactory controls. It should be noted that viable organisms are necessary for a satisfactory test, requiring that old strains be subcultured to obtain fresh cultures.

Animal virulence tests were performed with 433 of the cultures by subcutaneous inoculations of suspensions of the organisms into the groins of guinea pigs. The inocula consisted of either a 1 ml. sample of the sediment of the sodium hydroxide-treated specimen, or a suspension of the culture standardized with a Hopkins vaccine tube (3), and diluted to obtain the desired concentration. With pure cultures, dosages of 0.1 to 5.0 mg. were used throughout. Animals surviving at the end of eight weeks were sacrificed and the extent of infection was recorded according to the distribution of macroscopic lesions. All lesions were

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examined microscopically to demonstrate the presence or absence of acid-fast bacilli. Animals were reported as negative for tuberculosis if there was no involvement of the lymph nodes, spleen, liver, or lung.

Colonies of *Mycobacterium* were differentiated according to their colonial characteristics (4) as either human-type tubercle bacilli (dry, friable, somewhat rough, giving a "cabbage" appearance) or acid-fast saprophytes (soft, creamy, visually smooth, and chromogenic).

## RESULTS

As shown in table 1, the results obtained with the dye reaction procedure on 327 "wild" strains of *Mycobacterium* isolated from routine pathologic material were in complete agreement with the identification of the organisms, using colony characteristics as criteria. In no instance did an acid-fast saprophyte fail to reduce the dyes, and none of the 322 cultures of *M. tuberculosis* decolorized the dyes. None of the specimens in this series was inoculated into animals.

The results obtained with 433 cultures for which dye reaction, colony characteristics, and guinea pig virulence were compared are presented in table 2. No reduction of dye occurred with 305 cultures. Of these, 299 (98.1 per cent) produced progressive tuberculosis in guinea pigs. One culture was identified as *M. tuberculosis* on the basis of colony characteristics. Although this organism produced involvement only of the iliac and inguinal lymph nodes, it failed to reduce the dyes. Another culture, with all the colonial characteristics of human-type *M. tuberculosis*, was avirulent for guinea pigs and did not reduce the dyes. The culture isolated from a lesion produced by BCG also produced no pathologic involvement when inoculated into guinea pigs. Finally, there were three cultures which contained rough, orange colonies. These organisms were avirulent for guinea pigs and failed to reduce the dyes.

Two of the cultures examined reduced the four dyes only slightly. One of these cultures consisted of smooth, white colonies avirulent for guinea pigs and chickens. The other culture contained smooth, white, hemispherical colonies which produced involvement of the spleen and lymph nodes when 5 mg. and 0.1 mg. dosages were inoculated into guinea pigs. These organisms were avirulent for rabbits and chickens.

Complete reduction of the dyes occurred with 128 cultures, of which 120 (93.7 per cent) were completely avirulent for guinea pigs. Seven of the cultures produced pathologic involvement limited to the iliac and inguinal lymph nodes

TABLE 1

RESULTS OF TESTS FOR DECOLORIZATION OF DYES BY 327 "WILD" STRAINS OF MYCOBACTERIA

| DYE REACTION* | COLONY CHARACTERISTICS | NUMBER |
|---|---|---|
| No reduction | *M. tuberculosis* (*hominis*) | 322 |
| Reduction | Acid-fast saprophyte | 5 |

* The four dyes used were phenol indophenol, sodium benzenoneindophenol, sodium benzenoneindo-3′-chlorophenol, and sodium 2,6-dibromobenzenone-indophenol.

TABLE 2

RESULTS OF GUINEA PIG INOCULATION AND OF TESTS FOR DECOLORIZATION OF DYES BY 433 "WILD" STRAINS OF MYCOBACTERIA

| DYE REACTION* | VIRULENCE FOR GUINEA PIGS | COLONY CHARACTERISTICS | REMARKS | NUMBER |
|---|---|---|---|---|
| No reduction | Progressive tuberculosis | *M. tuberculosis (hominis)* | | 299 |
| | Regional lymph nodes only | *M. tuberculosis (hominis* | | 1 |
| | Negative | *M. tuberculosis (hominis)* | | 1 |
| | Negative | *M. tuberculosis (hominis)* | BCG | 1 |
| | Negative | Acid-fast saprophyte | | 3 |
| Slight reduction | Negative | Acid-fast saprophyte | Avirulent for chicken | 1 |
| | Progressive tuberculosis | Acid-fast saprophyte | Avirulent for rabbit and chicken | 1 |
| Reduction | Progressive tuberculosis | Acid-fast saprophyte | Avirulent for rabbit | 1 |
| | Regional lymph nodes only | Acid-fast saprophyte | 1 strain avian type virulent for chicken | 7 |
| | Negative | Acid-fast saprophyte | | 120 |
| Total | | | | 435† |

* The four dyes used were phenol indophenol, sodium benzenoneindophenol, sodium benzenoneindo-3′-chlorophenol, and sodium 2,6-dibromobenzenone-indophenol.

† In two of the 433 cultures examined, subcultures showed two different colony types and these are tabulated as different organisms.

only after subcutaneous inoculations of rather large dosages (4 to 5 mg.). One of these latter cultures was identified as avian type *M. tuberculosis* since it was virulent for chickens. The avian colonies were smooth, white, and hemispherical. The last culture in this group consisted of smooth, white colonies. Guinea pigs inoculated with 1 mg. and 5 mg. dosages developed in six weeks a moderate degree of infection involving the regional lymph nodes and spleen. The culture was avirulent for rabbits inoculated intravenously with a 0.1 mg. dosage.

It is interesting to note the rather close correlation between dye reaction and colony characteristics in the present study. Of the 305 cultures which did not reduce the dyes, 302 (99.0 per cent) had colony characteristics typical of human-type *M. tuberculosis*. All of the 128 which reduced the dyes completely had colony characteristics typical of acid-fast saprophytes.

The comparison of colony characteristics with animal virulence is likewise of considerable interest. A total of 302 cultures had colony characteristics typical of human-type tubercle bacilli. Of these, 299 (99.0 per cent) were capable of producing progressive tuberculosis in guinea pigs. Of the 133 cultures having characteristics of acid-fast saprophytes, 124 (93.2 per cent) were completely avirulent for guinea pigs, 7 (5.2 per cent) produced involvement only of the regional lymph nodes, and only 2 of the cultures produced progressive tuberculosis.

## DISCUSSION

The studies reported in the present paper were performed in an attempt to evaluate thoroughly the oxidation-reduction dye test as it might be used under routine conditions. The results of the dye reaction with 746 (98.1 per cent) of the cultures examined correlated completely with the identification of the organisms, based either on colony characteristics alone or on both animal pathogenicity and colony characteristics. With the remaining 16 cultures, there were discrepancies of varying degrees. Two of these cultures had colony characteristics typical of human-type tubercle bacilli. Although they both failed to reduce the dyes, one of them was completely avirulent for guinea pigs and the other was capable of infecting only the regional lymph nodes. It may be that these cultures were actually human-type *M. tuberculosis* which had become attenuated during prolonged subculture before they were sent to this laboratory for identification. If this is true, the organisms would be similar to H37Ra, which also does not reduce the dyes (1).

The findings with the culture isolated from the BCG lesion agree with the results obtained by Wilson and associates (1) when stock strains of BCG were tested. The results obtained with the three cultures having the colony characteristics of acid-fast saprophytes and yet failing to reduce the dyes are difficult to explain. In appearance, all three of these cultures resembled very closely the *M. phlei* used as a control in that they were rough and orange-colored. *M. phlei*, however, invariably reduces the dyes.

Equally difficult to explain is the culture composed of smooth, white, hemispherical colonies which reduced the dyes and yet was capable of producing an infection in guinea pigs involving the regional lymph nodes and spleen, even in only a 1 mg. dosage.

In the publication by Wilson, Kalish, and Fish (1), it is stated that an avian type (Kirchberg) of *M. tuberculosis* reduced the dyes. This finding has been confirmed in the present study with another avian strain.

Because of the discrepancies noted above, the dye test in its present form cannot replace routine animal inoculation. Nevertheless, the results obtained indicate that the test may be of value as an aid in the laboratory diagnosis of tuberculosis.

## SUMMARY

The oxidation-reduction dye reaction has been used in this laboratory to test a total of 1,033 cultures of acid-fast bacilli. The dyes used were phenol indophenol,

sodium benzenoneindophenol, sodium benzenoneindo-3′-chlorophenol, and sodium 2,6-dibromobenzenone-indophenol.

In 327 strains, there was 100 per cent agreement between results of the dye reaction and the colony characteristics. There was 97.7 per cent agreement in a total of 706 strains when dye reaction, colony characteristics, and guinea pig virulence were compared.

In its present form, the dye test cannot replace animal inoculation as a measure of the animal pathogenicity of mycobacteria.

SUMARIO

*Valuación de la Prueba de Oxidación-Reducción del Colorante para la Determinación de la Virulencia de las Micobacterias in Vitro*

En el laboratorio de los AA. han usado la reacción de oxidación-reducción del colorante para estudiar un total de 1,033 cultivos. Los colorantes usados fueron: indofenol fénico, bencenonoindofenol sódico, bencenonoindo-3′-clorofenol sódico y 2,6-dibromobencenonoindofenol sódico.

Al comparar la reacción del colorante y las características de las colonias, hubo un acuerdo de 100 por ciento en 327 cepas. Al comparar la reacción del colorante, las características de las colonias y la virulencia en el cobayo, hubo un acuerdo de 97.7 por ciento en un total de 706 cepas.

En su forma actual, la prueba del colorante no puede suplantar a la inoculación en animales.

RESUME

*Evaluation du test coloré d'oxydation-réduction pour la détermination in vitro de la virulence des Mycobactéries*

La réaction colorée du phénomène d'oxydation-réduction a été utilisée par les auteurs pour déterminer la virulence de 1.033 cultures. Les colorants utilisés étaient le phénol indophénol, benzénoneindophénol de sodium, le benzénoneindo-3′-chlorophénol de sodium, et le 2,6-dibromobenzénone-indophénol de sodium.

Pour 327 souches, les tests sont en accord dans la proportion de 100%, quand la réaction colorée et les caractéristiques de la colonie ont été comparées. Sur un nombre total de 706 souches la proportion est de 97, 7% quand la réaction colorée, les caractéristiques de la colonie et la virulence pour le cobaye sont comparées.

Dans sa forme actuelle, le test de coloration ne peut remplacer l'inoculation à l'animal.

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# GROWTH STUDIES ON CHROMOGENIC ACID-FAST BACILLI, WITH PARTICULAR EMPHASIS ON THEIR OXYGEN REQUIREMENTS[1]

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## INTRODUCTION

The increase in the number of chromogenic acid-fast bacilli isolated since Pinner's studies in 1935 (1) has led to a renewed interest in the role which these organisms play in the pathologic processes from which they have been isolated. Chromogenic acid-fast bacilli have been cultured from tuberculous and non-tuberculous processes in various parts of the body as well as from nonclinical sources.

Pinner (1), in his review of the early work on these organisms, cites several examples of their isolation from nonclinical sources such as butter, water faucets, and tap water. Indeed, Segard and Thompson (2) reported the isolation of chromogens from the sodium hydroxide and chlor-phenol-red solutions used in their concentration procedure for the isolation of tubercle bacilli from clinical specimens.

Chromogenic acid-fast bacilli have been isolated in 1 to 4 per cent of diagnostic specimens sent in for examination for tubercle bacilli (3). They have been isolated along with the tubercle bacillus from sputum, urine, blood, and gastric lavage specimens in cases of tuberculosis of the lung and kidney and have been recovered by themselves from cases of atypical pneumonitis, lung abscess, chronic nephritis, and apparent pulmonary tuberculosis (1, 4–10).

Most of the previous investigators have studied the growth of these organisms on media adapted for the culture of *Mycobacterium tuberculosis* and have found that the growth obtained was comparable to that observed with the tubercle bacillus. Virtually all of the strains used by other workers, as well as those in the present investigation, were isolated on such media. Hence, for the most part, they were studied under aerobic conditions. Tarshis and Frisch (3) have found that they could separate their organisms on the basis of growth in thioglycollate broth. However, even those of their strains which did grow in this medium took a long time to develop.

In the studies to be described, in which eight strains of freshly isolated chromogenic acid-fast bacilli were used, the primary interest has been in the thioglycollate medium, in which good micro-aerophilic growth was obtained in eighteen to twenty-four hours with a moderate inoculum.

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## MATERIALS AND METHODS

The strains used in these studies were all freshly isolated from the following sources:

| STRAIN NUMBER | SPECIMEN | CLINICAL DIAGNOSIS |
|---|---|---|
| 613 | Urine | Ulcerated bladder |
| 617 | Sputum | Lobar pneumonia |
| 634 | Sputum | History of apparent pulmonary tuberculosis of long standing |
| 645 | Urine | Acute cystitis |
| 650 | Sputum | Consolidation followed by pleural thickening of the right upper lobe of the lung |
| 662 | Sputum | Fatal bronchogenic carcinoma |
| 664 | Biopsy | Ulcer on the right groin |
| 666 | Sputum | Apparent pulmonary tuberculosis |

No typical tubercle bacilli were isolated from any of these cases at the time they were seen.

In each instance the clinical specimen was concentrated, using oxalic acid, planted on Petragnani medium, and incubated at 37°C. Smears were made and stained from the original and the concentrated specimens.

Cells from the primary cultures were emulsified in saline and standardized turbidimetrically to approximately $1 \times 10^8$ cells per ml.; one-tenth ml. aliquots were then inoculated into tubes of the media given in table 1. The solid media were all slanted in 20 mm. screw-cap tubes. The thioglycollate broth (10 ml. in 16 mm. screw-cap tubes) was inoculated by placing the tip of a 1 ml. pipette containing the cell suspension at the bottom of the tube to be inoculated and then raising it to the top as the 0.1 ml. was released. Acid-fast stained smears were made from all of the cultures.

The role of pH in the observed growth on the Sabouraud dextrose medium was ascertained by comparing the response on the medium as normally prepared (pH 5.6) to that on the medium adjusted to a pH of 7.0.

The effects of glycerol and serum (beef) were tested by adding these constituents to the thioglycollate broth to final concentrations of 5 per cent and 1 per cent, respectively. The serum was sterilized by filtration and added aseptically to the sterile medium.

The thioglycollate medium (fluid thioglycollate, Difco) was composed of:

| | | |
|---|---|---|
| Casitone | 1.5 | gm. |
| Yeast extract | 0.5 | gm. |
| Dextrose | 0.5 | gm. |
| Sodium chloride | 0.25 | gm. |
| L-Cystine | 0.075 | gm. |
| Thioglycollic acid | 0.03 | ml. |
| Agar | 0.075 | gm. |
| Resazurin, certified | 0.1 | mg. |
| Distilled water | 100.0 | ml. |

The components in this medium necessary for growth were determined by the cumulative stepwise deletion of the constituents as given by the following protocol:

No. 1. Fluid thioglycollate (Difco dehydrated)
No. 2. The same formula as No. 1 but prepared from the undivided constituents
No. 3. Medium No. 2 less thioglycollate
No. 4. Medium No. 3 less the cystine
No. 5. Medium No. 4 less the agar

No. 6. Medium No. 2 less the dextrose
No. 7. Medium No. 2 less the yeast extract

This latter study was performed some time after the first experiments were finished; by this time the strains had been subcultured several times on Petragnani or Löwenstein medium. It was noted that heavier cell suspensions were required to achieve good growth in the thioglycollate medium and that the growth thus obtained was still not comparable to that observed when the inocula were prepared from the freshly isolated strains.

## RESULTS

Smears, stained by the Ziehl-Neelsen technique, from the specimens as well as the cultures revealed these organisms to be quite similar to the tubercle bacillus. They tended, however, to be larger and more granular and were not consistently acid-fast, particularly in young cultures. Branching forms were seen, but they occurred rarely. On primary isolation, they appeared as discrete, highly convex, pigmented colonies which had even peripheries and butyrous consistencies. The organisms were easily emulsified in saline to give even suspensions. The colonies initially were pale yellow to orange-yellow in color and then darkened as the cultures aged. Strains 613, 634, and 666 were pale yellow; the remainder were orange-yellow.

The chromogens studied grew as well on Sabouraud dextrose agar as they did on Petragnani medium and as well at 25°C. as at 37°C. (table 1). Pigmentation was darkest on Löffler medium, less so on Sabouraud dextrose agar, even less on Petragnani medium, and least on the nutrient agar-yeast extract medium. Microscopically, the organisms appeared as highly beaded, acid-fast as well as non-acid-fast rods. On a given slide the number of acid-fast cells varied between 20 and 100 per cent. Organisms from older cultures tended to be more consistently acid-fast. The microscopic appearance of these organisms when grown in the thioglycollate medium may be seen from figure 1.

The most striking result to be seen in table 1 was the rapid growth in the thioglycollate medium. In this medium, growth was greatest at the interphase between the nonoxygenated portion of the tube and the area directly above it into which oxygen had diffused. There was little, if any, evidence of growth at the bottom of the tube or in the oxygenated layer at the top (figure 2). The better response on Sabouraud dextrose agar as contrasted to the nutrient agar-yeast extract medium was not due to the low pH of the former since adjusting the Sabouraud medium to a pH of 7.0 did not result in decreased growth (table 1).

The addition of 1 per cent serum or 5 per cent glycerol to the thioglycollate medium had no stimulatory action; if anything, glycerol had a slight inhibitory effect.

From the results in table 2, it can be seen that the removal of thioglycollic acid and agar rendered the medium unsuitable for the rapid growth of the chromogens. The presence of agar apparently masked part of the effect due to the removal of thioglycollate alone from the medium. L-Cystine and probably yeast extract were somewhat inhibitory; dextrose, however, did have a stimulatory effect on the organisms. "Interphase growth," as described previously, occurred in all of those tubes in which visible turbidity was noted.

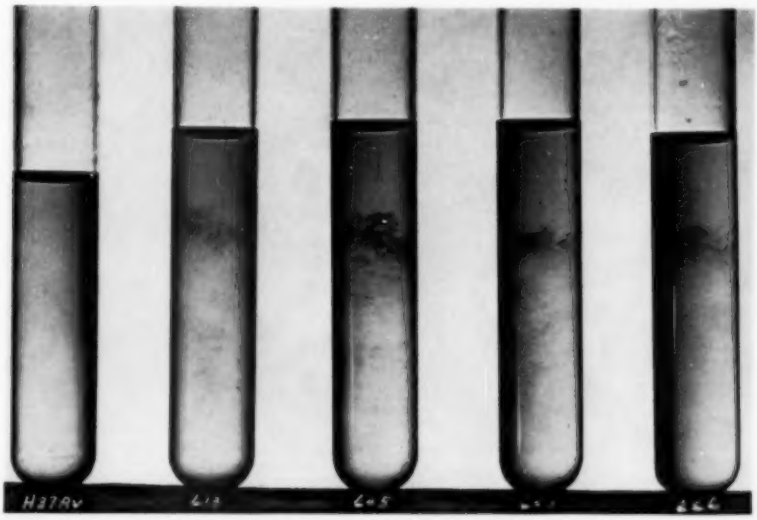

FIG. 1. The "interphase growth" of chromogenic acid-fast organisms in fluid thioglycollate broth after thirty-six hours' incubation at 37°C. The tubes were inoculated by the method indicated in the text. The dark area above the flocculent, stringy, growth was due to recoloration of the Eh indicator resazurin. By the time this photograph was taken, the strains had undergone several subcultures on Petragnani medium; growth was more abundant and appeared more rapidly in earlier studies in which the inoculum came from the primary culture.

TABLE 1

THE TIME OF APPEARANCE OF GROWTH IN VARIOUS MEDIA AT 37°C. AND AT 25°C.

| MEDIUM | TEMPERATURE OF INCUBATION | STRAIN | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 613 | 617 | 634 | 645 | 650 | 662 | 664 | 666 |
| | °C | | | | | | | | |
| Petragnani | 37 | 8 | 6 | 6 | 6 | 8 | 12 | 8 | * |
| Petragnani | 25 | 6 | 12 | 8 | 8 | 8 | 12 | * | * |
| Sab | 37 | 8 | 8 | 8 | 8 | 12 | 8 | 6 | * |
| Sab | 25 | 6 | 6 | 6 | 6 | 6 | 10 | * | * |
| Sab (pH 7.0) | 37 | 6 | 6 | 6 | 8 | 6 | 8 | * | * |
| NaY | 37 | — | — | — | — | — | — | * | * |
| NaY | 25 | 8 | 20 | 20 | 20 | 20 | 20 | * | * |
| Löffler | 37 | 11 | 11 | 11 | 11 | — | 11 | * | * |
| Thio | 37 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

The numbers indicate the days needed for good growth to appear. *, not done. —, no growth in 30 days. Sab—Sabouraud dextrose agar; NaY—nutrient agar +1 per cent yeast extract; Thio—fluid thioglycollate (Difco).

FIG. 2. Microscopic appearance of chromogenic acid-fast bacilli. Ziehl-Neelsen stain of cells from a thioglycollate culture; strain 645. (× 1,200)

TABLE 2

THE COMPONENTS OF THIOGLYCOLLATE MEDIUM NECESSARY FOR GROWTH

| STRAIN | MEDIA* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. 1 | | No. 2 | | No. 3 | | No. 4 | | No. 5 | | No. 6 | | No. 7 | |
| | 12 hours | 48 hours | 12 hours | 48 hours | 12 hours | 48 hours | 12 hours | 48 hours | 12 hours | 48 hours | 12 hours | 48 hours | 12 hours | 48 hours |
| 613 | ++ | ++ | ++ | ++ | + | + | + | ++ | 0 | 0 | + | ++ | + | + |
| 645 | + | ++ | + | ++ | + | + | + | ++ | 0 | 0 | + | + | + | ++ |
| 617 | ++ | ++ | ++ | ++ | + | ++ | + | ++ | 0 | 0 | ++ | ++ | ++ | ++ |
| 634 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | 0 | 0 | ++ | ++ | ++ | ++ |
| 650 | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ | 0 | 0 | + | ++ | ++ | ++ |
| 662 | + | ++ | + | ++ | + | + | + | ++ | 0 | 0 | + | ++ | + | ++ |
| 664 | + | ++ | + | ++ | + | + | ++ | ++ | 0 | 0 | + | ++ | ++ | ++ |
| 666 | ++ | ++ | ++ | ++ | + | + | + | ++ | 0 | 0 | + | ++ | ++ | ++ |
| Avian† | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H37Rv | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Media: No. 1.—Fluid thioglycollate (Difco); No. 2.—same formula as No. 1 but prepared from the individual components; No. 3.—medium No. 2 less the thioglycollate; No. 4.—medium No. 3 less the L-cystine; No. 5.—medium No. 4 less the agar; No. 6.—medium No. 2 less the dextrose; No. 7.—medium No. 2 less the yeast extract.

† Avian—Van Es strain of the avian tubercle bacillus.

0 = no growth; + = moderate growth; ++ = good growth.

## DISCUSSION

It should be emphasized that the results presented in table 1 were obtained with inocula from freshly isolated strains and that their ability to grow in the thioglycollate medium decreased following successive subcultures on egg-glycerol-potato medium. For this reason, stock chromogenic strains used by other investigators were not utilized in these studies.

Since growth occurred micro-aerophilically on rather simple media, the critical factor for the rapid development of these chromogens was apparently the oxygen tension of the medium. Because of their inability to grow rapidly in the absence of thioglycollate and agar and because of their "interphase growth," the chromogens studied might be looked upon as micro-aerophiles in respect to their oxygen requirements. In fact, as their ability to grow in the thioglycollate medium decreased on subculture, this might be the form found in the body.

While chromogenic acid-fast bacilli do resemble the tubercle bacillus in their staining reactions, in their growth stimulation by dextrose, and in their inhibition by agar in the medium (0.15 per cent exerts a slightly toxic effect, unpublished data), they do differ markedly in other respects: they are pigmented; they grow on simple media; they are micro-aerophilic; and they are weak pathogens (1, 3). In view of the above discussion and in view of the ability of chromogenic acid-fast bacilli to grow and produce some branching forms on Sabouraud dextrose agar, one might consider them to be *Mycobacterium-Nocardia* intermediates. In fact, Cuttino, McCabe, and Weil (7) have classified a chromogenic acid-fast bacillus, isolated from a case diagnosed as lymphosarcoma, as a *Nocardia*. The organism as described by them was quite similar, both culturally and microscopically, to those used in these studies with the exception that branching forms were more common in their strain.

Pathogenicity studies are now in progress using these strains as well as several others obtained since these experiments were completed.

## SUMMARY

The growth characteristics of eight strains of chromogenic acid-fast bacilli freshly isolated from human sources were studied. It was shown that they all grew as well on Sabouraud dextrose agar as on egg-glycerol-potato medium and that they grew as well at 25°C. as at 37°C. Furthermore, it was demonstrated that growth was obtained in liquid thioglycollate medium in eighteen to twenty-four hours. By the stepwise elimination of the components of the thioglycollate medium and by the type of growth obtained, it was shown that these organisms are micro-aerophilic in their oxygen requirements and that the oxygen tension of the medium is the important factor for the rapid growth of the organisms. The ability to grow rapidly from small inocula in the thioglycollate medium decreased with repeated subculture on the media usually used for the cultivation of the tubercle bacillus.

The results were discussed in regard to the classification of these organisms.

SUMARIO

*Estudios del Crecimiento en Bacilos Acidorresistentes Cromógenos Recalcando en Particular los Requisitos de Oxígeno*

Este estudio de las características del crecimiento comprendió ocho cepas de bacilos ácidorresistentes cromógenos, recién aislados de seres humanos. Demostróse que todos crecían igualmente bien en el agar-dextrosa de Sabouraud como en el medio de huevo-glicerina-patata y que crecían lo mismo a 25° como a 37°C. Además, se observó que se obtenían colonias en medio de tioglicolato líquido en dieciocho a veinticuatro horas. Mediante la eliminación gradual de los componentes del medio de tioglicolato y por la clase de colonias obtenidas, se demostró que dichos gérmenes son microaerófilos en sus requisitos de oxígeno y que la tensión del oxígeno del medio constituye el factor de importancia para la rápida proliferación de los microbios. La capacidad para desarrollarse rápidamente de pequeños inóculos en el medio de tioglicolato disminuyó al hacerse subcultivos repetidos en los medios empleados habitualmente para el cultivo del bacilo tuberculoso.

Discútense los resultados con respecto a la clasificación de estos microorganismos.

RESUME

*Recherches culturales sur les bacilles chromogènes acido-résistants en insistant sur leurs besoins en oxygène*

Les caractéristiques culturales de huit souches de bacilles chromogènes, acido-résistants, fraichement isolées chez l'homme sont étudiées. On a observé qu'elles poussaient aussi bien sur le milieu dextrose-agar de Sabouraud que sur le milieu oeuf-glycérol-pomme de terre et qu'elles se développaient aussi bien à 25°C. qu'à 37°C. De plus, il a été démontré que la culture se développait dans un milieu liquide au thioglycolate en dix-huit à vingt-quatre heures. A la suite de l'élimination graduelle des divers constituants du milieu au thioglycolate et selon le type de culture obtenu, il a été démontré que ces organismes étaient microaérophiles en ce qui concerne leurs besoins en oxygène et que la tension de l'oxygène dans le milieu représentait un important facteur dans la rapidité de développement des cultures de ces souches. La capacité de développement rapide à partir d'un petit inoculum dans le milieu au thioglycolate diminuait par la répartition du repiquage sur les milieux habituellement utilisés pour la culture du bacille tuberculeux.

Les auteurs commentent ces résultats par rapport à la classification de ces bacilles.

*Acknowledgments*

The writers would like to express their appreciation to Dr. Carol Popp, formerly of the Missouri State Sanatorium; and to Drs. C. Leech, J. Allee, and K. Dietrich for making their records available to us. We also wish to thank Mr. James Crawford, formerly of the University Hospital, Columbia, Missouri, for his technical assistance.

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# *Case Reports*

## DESTRUCTIVE PRIMARY TUBERCULOSIS OF THE FAUCIAL TONSIL

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### INTRODUCTION AND REVIEW OF THE LITERATURE

The present report is based on an experience with 4 cases of destructive, tuberculous inflammation of the faucial tonsil in which the tonsil would appear to have been the site of a primary infection. In the absence of comprehensive post-mortem examinations, it is not possible to establish with certainty the primary nature of the tonsillar infection in any one of these cases. Nevertheless, in all of the cases the available evidence points in the direction of a primary tonsillar infection and offers no support for the contrary view.

It has long been recognized that the tonsil is commonly the site of a tuberculous inflammation, and many careful histologic and bacteriologic studies have been made on tonsils removed from unselected patients, from patients known to be suffering from tuberculous cervical adenitis, and from patients suffering or recovered from other tuberculous infections. There is, however, a general agreement that tuberculosis of the tonsil, whether primary or secondary to an infection established elsewhere in the body, is seldom other than clinically occult.

Long, Seibert, and Gonzalez (1) note that the condition, although common, is seldom recognized clinically. In reference to the large number of patients suffering from active tuberculosis of the adult type examined annually at the Henry Phipps Institute, they remark that "tuberculosis of the tonsils is almost never recognized macroscopically." Long and his associates also refer to cases recorded by Suve and by Hamperl and Wallis which appeared to be examples of primary infection of the tonsil satisfying rigorous criteria. No reference is made to the tonsillar lesion in these cases being destructive and, unfortunately, the case records are not available to the present writer.

Webster (2), in a study of tonsils removed from children suffering from tuberculous cervical lymphadenitis, found tuberculous tonsillar lesions in 46.5 per cent. He remarked that tuberculous infection of the tonsils rarely advances beyond the microscopic stage in contrast to the caseous softening so often seen in the closely related cervical lymph nodes. Rosencrantz and Hurwitz (3) reported a series of nineteen children ranging in age from sixteen months to twelve years in whom microscopic examination of adenotonsillar tissue showed tuberculosis. They noted that caseation was not a marked feature and that the surface epithelium of the tonsil was invariably intact. In all of these children, roentgenographic examination of the chest showed enlarged hilar lymph nodes, pulmonary infiltrations limited to one lobe, or pleural effusion. In two instances only did they record enlargement of cervical lymph nodes. In all cases, therefore, in this group there was suggestive evidence of a primary infection of the lungs. This view is supported by the finding of gastric contents positive for *M. tuberculosis* on guinea pig inoculation in eighteen of the nineteen cases in spite of the integrity of the surface epithelium of the tonsils noted by the writers. Moreover, they stress the fact that a preoperative diagnosis of tuber-

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culosis of the tonsils was impossible in their cases because there was no clinical evidence of a tonsillar lesion. They further note that during a period of many years no clinically recognizable case of tonsillar tuberculosis among children had been observed by them.

Bernstein (4) reported four cases of tuberculosis of the tonsil but described the tonsillar involvement as of the "latent" type. In two cases, tonsils which did not appear to be abnormal clinically were enucleated because of the persistence of tubercle bacilli in the sputum in the face of satisfactory resolution of pulmonary disease. In a third case, the tonsils were removed to obtain biopsy material in a patient with bilateral pulmonary infiltrations and hilar adenopathy suspected to be suffering from Boeck's sarcoid. In the fourth case, hypertrophied tonsils were removed following complaint of frequent, persistent, recurrent sore throats some two and one-half years after a diagnosis of pulmonary tuberculosis had been established.

Hudson and Woollaston (5) reported a case of massive tuberculosis of the tonsils, but in this instance frank, progressive tuberculosis of the lungs, with sputum positive for *M. tuberculosis*, was present before there was any evidence of tuberculous involvement of the tonsils or cervical lymph nodes. In this case there was nothing to suggest a primary tonsillar infection and much to suggest that the destructive lesion of the tonsils was a relatively late secondary manifestation.

Mirande (6) reported the case of a patient with two months' history of dysphagia showing almost complete destruction of the left tonsil and ulceration extending to the neighboring faucial pillars. The abstracted report indicates that microscopic examination revealed tuberculosis of the tonsils notwithstanding the fact that no further tuberculous lesion could be found elsewhere, such as lungs, intestinal tract, or lymph nodes. The specifically noted absence of lymph node lesions in this case must raise a certain doubt concerning the primary nature of the tonsillar infection.

The case reported by Furman (7) under the title of "Primary Ulcerated Infiltrative Tuberculosis of the Tonsils, Velum and Pharynx" has nothing in common with the cases reported in the present paper. In view of the roentgenographic findings of "numerous calcified nodes opposite both hilar and several calcified tubercles near the right base," it is difficult to accept the multiple oropharyngeal ulcerations as a manifestation of a primary tuberculous infection.

In the many articles on the subject which have been consulted, occasional reference is made to isolated case reports of destructive tuberculous lesions of the tonsil which have been regarded as the outcome of primary tonsillar infections. Unfortunately, the resources available to the present writer have failed to provide either the original case reports or detailed abstracts.

## CASE REPORTS

*Case 1.* (A.R.P.). The patient, a 23-year-old Maori female, was moribund when first seen in consultation in July, 1941. She was emaciated and suffering from a terminal enteritis. The most interesting clinical features in view of her history related to the throat and neck. The left tonsillar fossa was involved in an ulcer with sloughing base extending upward to the soft palate, backward on to the lateral wall of the pharynx, and downward to the base of the tongue, left vallecula, and lateral aspect of the left aryepiglottic fold. There was no evidence of an intrinsic tuberculous laryngitis. A chain of enlarged, firm, lymph nodes running down from below and behind the angle of the jaw was present on the left side of the neck. The breath was heavily tainted with a sweetish, musty odor reminiscent of the diphtheritic throats of an earlier experience in isolation wards.

The writer is indebted to Dr. C. H. King of Auckland for a note on the earlier history of this patient who had been in a sanatorium under his care. She had been operated upon

at the age of three years for removal of tonsils and adenoids. Toward the end of 1940, she began to complain of persistent sore throat and, in December of the same year, noticed enlarged nodes in the left side of her neck. She continued with her housework until February, 1941, when she developed marked lassitude, night sweats, cough, and scanty expectoration. A roentgenogram of the chest taken at this time showed a miliary infiltration of the upper lung fields.

On admission to the sanatorium on April 21, 1941, the patient had a hectic temperature, and her sputum was positive for *M. tuberculosis*. Dr. King noticed an ulcer on the left tonsil covered with a whitish exudate or slough. Obviously, the left tonsil had, in part or whole, been spared in the earlier operation. He confirmed the presence of enlarged lymph nodes in the left anterior cervical chain. The patient deteriorated rapidly with loss of weight, diarrhea, and dysphagia; on June 26, 1941, was discharged from the sanatorium at her own request.

*Case 2.* (V. McI.). The patient, a 14-year-old female, was first seen on December 31, 1941. She was referred by Dr. W. McKechnie, who was then Medical Superintendent of the Mangonui County Memorial Hospital. There was no early history of moment and no family or contact history of tuberculosis. Her illness began some three or four months earlier with the complaint of more or less persistent sore throat. At some time during this period she developed a painless swelling in the neck on the left side. Early in December, 1941, the tumor in the neck increased considerably in size and Dr. McKechnie found what he considered to be an acute bilateral follicular tonsillitis. The right tonsil responded promptly to treatment, including sulfanilamide by mouth, but the left tonsil continued to enlarge until it reached the midline. Dr. McKechnie decided then to incise what he thought to be a quinsy but found in his own words "a foul necrosis of the tonsil which I viewed with suspicion." A specimen of the necrotic tissue was forwarded to Dr. E. F. Fowler of Auckland, who reported: "sections show a tuberculous lesion with a marked superadded acute inflammatory infiltration." Following operation the left tonsil rapidly reduced in size.

On the third postoperative day the patient developed a number of raised, reddish-purple, slightly tender swellings, approximately one inch in diameter, on the anterior aspects of the legs and some similar, though smaller, swellings on the upper limbs. Dr. McKechnie described these as "emphatic, definite and characteristic erythema nodosum." Residual pigmentation from this eruption was still present when the patient was first seen by the present writer.

The right tonsil was unremarkable in appearance, but the left tonsil was still moderately enlarged with a coarse granular surface not obviously ulcerated. The breath again was curiously tainted and called to mind an impression one had retained from the first case in this series. There was a single lymph node the size of a small walnut below and behind the angle of the jaw on the left side. There were no abnormal findings in the chest, and a chest roentgenogram obtained on admission to the hospital was normal.

On January 7, 1942, the left tonsil was enucleated by Mr. J. Hardie Neil in the Auckland Hospital. The histologic report states baldly that "the specimen shows an extensive tuberculous process."

The tonsil fossa healed promptly and, when the patient was next seen on February 20, 1942, she felt generally well, had no discomfort in the throat, and the enlarged lymph node in the neck was reduced in size and firmer in consistency. She then returned to Dr. McKechnie's care. In May, 1942, because of recurring anxiety about the right tonsil, he decided on an enucleation and submitted the specimen to Dr. Fowler who reported: "Sections show a tuberculous lesion. There are numerous tuberculous follicles with giant cells." Again, the tonsil fossa healed promptly. It should be noted that there was never any suggestion of cervical adenitis on the right side.

On February 2, 1942, the patient was very well. The throat appeared to be quite healthy, and it was not possible to palpate any enlarged lymph nodes in the neck on either side. A roentgenogram of the chest again revealed no abnormalities.

Recurrent enlargement of lymph nodes at the original site in 1944 responded to deep X-ray therapy. Following a further recurrence in 1947, Mr. Douglas Robb did a restricted block dissection of the left anterior cervical nodes after a further short course of deep X-ray therapy. The report on the specimen from the Pathological Department, Auckland Hospital, reads: "Specimen consists of a mass of caseous glands. Microscopically, it shows active tuberculous adenitis." Neither direct examination, nor culture, nor guinea pig inoculation demonstrated *M. tuberculosis*, but the recent irradiation may well have been responsible for this. There had been no further incident when the patient was last seen in 1952.

*Case 3.* (D. C.). The patient, a 20-year-old female, was first seen on January 6, 1942, complaining of slight unproductive cough during the previous three to four months. She ascribed this to "a feeling as of something in the throat." Approximately two months previously she had noticed a swelling in the left side of her neck. She reported this swelling to her own doctor, who told her that the left tonsil was "nasty." When the swelling failed to subside, a roentgenogram of the chest was obtained and this disclosed a miliary infiltration of right and left upper zones.

The significant findings on examination were confined to throat, neck, and chest. The left tonsil was grossly enlarged, with an irregular surface studded with yellowish, submucosal nodules of the size of small match heads. The tonsil felt "craggy" on palpation. There was no tenderness. The breath was curiously tainted. The hypopharynx and larynx were normal.

Below and behind the angle of the jaw on the left side there was a group of enlarged lymph nodes. There was slight impairment of percussion note over both upper zones of the chest, without recognizable muscular wasting, altered breath-sounds, or moist sounds.

This patient was referred to Mr. J. Hardie Neil, who enucleated both tonsils on January 27, 1942. Histologic examination of the left tonsil showed extensive caseous tuberculosis. When next seen on June 3, 1942, after four months of bed rest she was generally well, though still complaining of some irritation of left side of throat. The left posterior faucial pillar was thickened, and the mucosa of the lateral pharyngeal wall immediately posterior to the pillar was indurated and superficially ulcerated. There was no longer any palpable enlargement of lymph nodes in the neck. Temporary relief was afforded by fulgeration, but the pharyngeal ulceration recurred and by March, 1943, the associated infiltration extended downward to involve the pyriform fossa and left aryepiglottic fold. The roentgenographic abnormalities in the lung fields showed slight clearing since the original examination. During a period of four weeks in the hospital, the patient deteriorated rapidly, with increasing pain in throat and dysphagia; she died shortly after removal to her own home.

*Case 4.* (I. McD.). The patient, a 9-year-old Maori girl, was admitted to the hospital on October 9, 1944, with a note from her own doctor giving a history of sore throat, cough, loss of condition, and swollen nodes in neck for the previous five weeks. The child herself was shy and silent. Her mother, already in the hospital suffering from advanced pulmonary tuberculosis, knew nothing of the child's illness. She was febrile on admission. There was no significant abnormality on physical examination of the chest, although roentgenographic examination the following day showed a coarsely stippled infiltration throughout both lung fields without any suggestion of hilar or mediastinal lymphadenopathy.

The left tonsil was unremarkable. The right tonsil was grossly enlarged, with a craggy pharyngeal surface. Lymph nodes were enlarged and readily palpable on both sides of the neck but more especially on the right side from the angle of the jaw downward. She did not produce any sputum and, as she was extremely miserable, stomach washings were not taken.

Her course was rapidly downhill, with hectic fever and increasing discomfort in the throat discouraging thorough examination. On November 15, however, the right tonsil was obviously breaking down as the result of a caseous necrosis, and the neighboring structures in the pharynx were edematous and covered with sticky mucopus. Unfortunately, no swab

was taken from the throat. Early in December a fluctuant softening of matted lymph nodes in the right side of the neck developed. She died on December 11, some nine weeks after admission. Partly from consideration for the child, partly on an understanding from the mother that an autopsy would be granted, attempts to obtain bacteriologic and histologic confirmation were not pressed. Despite the mother's assurances, autopsy was refused by dominant relatives.

## DISCUSSION

These 4 cases conform to a pattern which marks them as unique in the writer's experience of more than twenty years' association with tuberculosis in hospitals and private practice. All of the patients were young, and the initial symptom was soreness in the throat followed by enlargement of the anterior cervical lymph nodes as the first objective sign of disease. In the 3 older patients, the lymph node enlargement was confined to the side of the neck related to the tonsil responsible for the initial symptom. In the youngest patient, the position was obscured by minor bilateral enlargement of posterior cervical nodes, probably reflecting nonspecific upper respiratory infections so common in children. Even in this case, however, the dominant, progressive and necrotic adenitis was homolateral to the grossly diseased tonsil. In the 3 older subjects, certainly, and probably in the youngest, a considerable interval of time elapsed between the onset of throat soreness and the recognition of enlargement of the regional cervical lymph nodes. This interval is strictly in keeping with the classical picture of extension of a tuberculous parenchymal infection to the lymphatic pole of a primary complex. In all cases, palpable enlargement of superficial lymph nodes was confined to the neck and, with one exception, to the homolateral anterior cervical group.

In no case was there any history of an earlier tuberculous infection or any clinical or roentgenographic evidence of such involvement. Three patients had roentgenographic evidence of discrete, nodular (miliary) infiltration of the lung fields when first examined, but in no instance was there any suggestion of hilar or mediastinal lymphadenopathy or of pleural effusion. In the remaining case, the lung fields were clear throughout a prolonged period of observation. These findings suggest that the lung involvement, when present, reflected a dissemination through lymphatic or vascular channels from a primary focus elsewhere in the body. Finally, there were no symptoms or physical signs in any of the 4 patients to suggest a possible primary infection of the bowel. The enteritis suffered by one patient was a terminal event.

The question of treatment in cases of this nature should offer little or no difficulty in the present day with effective chemotherapeutic "cover" for any surgical intervention. Experience gained from the one case which presented before there had been any recognizable dissemination from the primary complex suggests that the treatment of choice would be enucleation of tonsils combined with an excision of enlarged regional lymph nodes under cover of antimicrobial therapy.

## SUMMARY

Four cases of caseous necrosis of the faucial tonsil are presented in which all of the available evidence suggests that the tuberculous inflammation of the tonsil was a primary lesion.

## SUMARIO

### *Tuberculosis Primaria Destructiva de la Amígdala Faucial*

Preséntanse 4 casos de necrosis caseosa de la amígdala faucial, en los que todos los datos disponibles indican que la inflamación tuberculosa de la tonsila era una lesión primaria.

RESUME

*Primo tuberculose destructrice de l'amygdale*

Quatre cas de nécrose caséeuse de l'amygdale sont présentés. Dans tous ces cas, les données dont on dispose suggèrent que l'inflammation tuberculeuse de l'amygdale constitue la lésion initiale.

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# TRANSMISSION OF GENITAL TUBERCULOSIS FROM HUSBAND TO WIFE VIA THE SEMEN[1]

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## INTRODUCTION

The transmission of genital tuberculosis from husband to wife by way of the semen has been reported infrequently. Only eight case reports have been found in a review of the literature. A summary of these cases and the present case is reported below in a revision of Hellerstrom's table from the French literature (1). It seems surprising that more men with tuberculous prostates do not infect their wives, in view of the fact that cultures of the semen of many of the writers' patients with prostatic tuberculosis have been positive for tubercle bacilli. More than 600 male patients, all of whom have viable tubercle bacilli in their urines, are under treatment on the writers' services, but only one case of genital transmission tuberculosis has been discovered.

## CASE REPORT

On March 16, 1949, a white housewife, age 33, came to the Mount Kisco Medical Group complaining of a small ulceration of the fourchette which was painful during urination. Two days later, she developed pain in the left groin, without fever or chills. A large tender lymph node was found in the left inguinal region at this time. After daily injections of 300,000 units of penicillin for four days, the lymph node in the groin seemed smaller and less tender, but the ulcer in the fourchette had grown larger and appeared to be forming an indolent ulcer with a granulating base. There was no redness or swelling around the ulcer. The enlarged lymph node in the left groin persisted. There were no palpable nodes in the right groin. Penicillin ointment failed to improve the ulcer. The total leukocyte count at this time was 5,050 per cu. mm., with neutrophils, 59 per cent; eosinophils, 3 per cent; lymphocytes 31 per cent; and monocytes, 7 per cent. The erythrocyte sedimentation rate was 34 mm. in one hour.

The patient had had two healthy children, born in 1942 and 1948 (one year previously), but the cervix was well healed and appeared entirely normal. Trichomonas vaginalis were found in a mild vaginal discharge. After four weeks the ulcerative lesion in the fourchette was larger and the inguinal adenopathy was still present. On April 13, 1949, the ulcer on the skin of the fourchette was completely excised for biopsy. The area healed primarily. The pathologic report of the biopsy material described only chronic granulation tissue. Tuberculosis was not suspected, and no smears or cultures for *M. tuberculosis* were made. The enlarged lymph nodes in the left groin then began to break down, forming an abscess with cellulitis of the overlying skin. This inguinal abscess was incised and drained. Cultures from the pus revealed no growth of ordinary pyogenic organisms. Cultures for *M. tuberculosis* were not taken. The draining sinus from the lymph nodes failed to heal during April, May, and June.

On May 3, 1949, because of the persistent drainage, a tuberculin test was done by the "patch" method and was negative. Two Frei tests were negative. On July 18, 1949, the tuberculin test was repeated, this time by the intradermal route with 0.1 mg. of Old Tubercu-

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lin. This gave a frankly positive reaction. During the next three months, the inguinal incision failed to heal. On August 6, 1949, preoperative chemotherapeutic preparation for excision of the sinus tract was begun. Dihydrostreptomycin, 1 gm. daily, and penicillin, 300,000 units, were administered twice daily for twenty-one days. After ten days of this treatment, the erythrocyte sedimentation rate was still 36 mm. in one hour. Surgery was deferred, but healing did not occur.

On October 25, 1949, a radical lymph node dissection of the left groin was done without additional dihydrostreptomycin. The wound healed per primum. The histopathologic diagnosis of the tissues removed was tuberculosis of two inguinal lymph nodes. No stains or cultures for *M. tuberculosis* were made. From this point on, the patient made an uneventful recovery. There was transient slight swelling of the left leg but this proved to be of no consequence. The ulcer in the fourchette has remained healed.

The patient's history showed no evidence of previous tuberculous infection and no known exposure to tuberculosis. She had never had a chest roentgenogram or tuberculin test prior to the present illness. Further inquiry into her personal history, however, revealed an interesting fact about her husband, whom she had married ten years previously.

### *Husband's History*

An abscess of the husband's left scrotum had been incised in 1944 during his Army service, five years before the diagnosis of tuberculosis in the wife and five years after their marriage. This scrotal abscess had drained for several months, and the patient was told that part of the epididymis had been removed. No mention of tuberculosis was made to the patient. In 1945, the husband was discharged from the Army and returned home. In 1947, the scrotum again became swollen and broke open following trauma of moderate degree. At this time the left epididymis was completely removed. The pathologic report of the excised epididymis described only chronic granulation tissue, and no search was made for tubercle bacilli at that time. The patient had no other symptoms.

The tissues which had been removed were subsequently recovered and restained with the knowledge that the wife's genital lesions were tuberculous. Acid-fast bacilli were found in the sections of the epididymis.

A second healthy child was born in 1948, after the epididymectomy. The first child had been born in 1942, before the first scrotal abscess.

In 1950, the husband was a healthy-appearing, successful young salesman, age 33, who had absolutely no complaints referable to the urinary tract, to the genitalia, or to his general state of health. He had had no urinary frequency or nocturia. He had a scar which resembled that of an epididymectomy on the left side of the scrotum. On palpation, his prostate gland was firm on the left side, suggesting tuberculous involvement. The right epididymis was normal. There was no kidney pain or tenderness.

Laboratory study of the husband revealed only an occasional leukocyte in voided urines of specific gravity 1.014; otherwise, the urinalyses were normal. The left ureteral specimen showed 3 to 5 pus cells per high-power field. Kidney function was normal.

Cystoscopic examination revealed a normal bladder without redness of the trigone or posterior urethra. Several small prostatic ducts were visible in the posterior urethra, with gray exudate extruding from one. Two tiny white "Pelouze" granules were also seen in the prostatic urethra. These findings were suggestive of prostatic tuberculosis. Retrograde pyelograms revealed a cavity, 1 cm. by 0.5 cm. in size, which ran laterally from the uppermost calyx of the left kidney. The outline of this cavity was only slightly irregular. The cavity appeared to lie in one of the lateral papillae of the upper calyx.

TABLE 1

CASES OF VENEREAL INFECTION WITH TUBERCULOSIS COMPILED BY HELLERSTROM (1), TO WHICH CASE OF OBRANT AND CASE OF PRESENT WRITERS HAVE BEEN ADDED

| AGE | SYMPTOMS | INCUBATION PERIOD | WOMAN'S SYNDROME RESEMBLED | MAN'S SYMPTOMS | TUBERCLE BACILLI FOUND IN | FOLLOW-UP | AUTHOR |
|---|---|---|---|---|---|---|---|
| 20 | Ulcer on fourchette; bilateral suppurating inguinal lymph nodes | 1 month for the adenitis | Lymphogranuloma inguinale | Orchid-epididymitis | Urine of husband and pus from wife's bubos | Disease regressed | Chevalier, Colin, Kaplan, and Ornstein, Bull. et mem. Soc. méd. d. hôp. de Paris, 1936, *60*, 2. |
| 22 | Ulcer at orifice of Bartholins gland; caseous inguinal adenitis | 4 weeks | Lymphogranuloma inguinale | TB kidney out 8½ months before the infecting coitus | Urine of man and pus from woman's bubo and ulceration | Woman in good health 3 years later | Hellerstrom, Acta dermat. venereol., 1937, *18*, 465 (Sweden) |
| 25 | Ulcer of hymen and inguinal nodes | Married 3 months | Lymphogranuloma inguinale or chancre | Husband had overt pulmonary TB | Ulcer and bubo of wife | Unknown | Urus, Zentralbl. f. Hautu Geschl. Kr., 1937, *58*, 125 (Turkey) |
| 19 | Ulcer of vulva, inguinal nodes, erythema nodosum | 2 months | Chancroid | Husband in good health | Ulcer and bubo of wife | Wife died of miliary TB 16 days after admission | Gate and Michel, Ann. de dermat. et syph., 1938, *9*, 665, Observation I. |
| 25 | Large ulcer fourchette and double suppurating inguinal lymphadenopathy | 1 month for the adenitis | TB of vulva | Husband died 1 month after marriage of rapid meningitis, probably tuberculous | Ulcer and bubo of wife | Wife healthy several weeks later | Gate and Michel, Ann. de dermat. et syph., 1938, *9*, 669, Observation II. |
| 19 | Peri chancre cruro-iliac bubos; erythema nodosum | Had had anal coitus with husband several times | Inguinal lymphogranuloma | Husband had had an epididymectomy 10 years after kick of horse | Koch bacilli in lymph nodes of woman | Unknown | Sezary, Horowitz et Walther, Bull. Soc. franc. de dermat. et syph., 1938, N: 07, p. 1352 |

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|---|---|---|---|---|---|---|---|
| 21 | Ulcer in fourchette and bilateral suppurating inguinal nodes | 3 to 4 weeks for the lymph nodes | Lymphogranuloma and soft chancre | Husband had bilateral epididymectomies 2 months before first coitus with this woman | Koch bacilli in wife's lymph nodes; husband's urine positive on guinea pig inoculation 2½ months after epididymectomy | Wife in good health 14 months later | Rochat, Ann. de dermat. et syph., 1929, p. 369. |
| 21 | Ulcer between clitoris and urethra—bilateral suppurating nodes | Nodes led to discovery of ulcer size of grain of rice | | | Husband's semen and urine positive by guinea pig inoculation; calcification in one kidney, nodules and cavities in prostate. Wife's ulcer and nodes positive for tubercle bacilli | Unknown | Obrant, Nord. med., 1951, *45*, 25 (Sweden) |
| 33 | Ulcer in fourchette; ulcerating bubo in left groin | About 3 weeks | Lymphogranuloma | Epididymectomy 5 years before; review of tissues revealed TB | Semen positive for tubercle bacilli by culture; voided urine positive by culture. Cavity in left kidney. Grandfather was tuberculous | Wife healthy after radical node dissection and dihydrostreptomycin; husband negative after 1 year of streptomycin and PAS | Lattimer, Colmore, Sanger, Robertson, and McLellan, 1954 (United States) |

It did not have the fuzzy, feathery, "moth-eaten" edges so characteristic of renal tuberculosis, and its appearance suggested that the lesion might be a healed tuberculous cavity with fairly smooth walls. No indentation could be seen on the kidney outline adjacent to this cavity.

Examination of the abdomen revealed no calcified mesenteric lymph nodes. Chest plates showed no evidence of tuberculosis, old or recent. The husband's family history revealed the fact that his grandfather, who raised him, had had a one-year illness, thought to have been tuberculosis, when the patient was five years old. The grandfather had pleurisy, a "collapsed lung," and was said to have recovered after one year of rest in Florida.

Culture of the husband's semen produced a luxuriant growth of tubercle bacilli. Culture of his voided urine gave a good growth of tubercle bacilli. Cultures from both ureteral specimens have been sterile on several occasions.

Treatment of the husband's prostatic and possible renal tuberculosis was started on January 16, 1951. It consisted of one year of multiple chemotherapy with streptomycin, 1 gm. in a single injection daily, and para-aminosalicylic acid (PAS), 12 gm. daily. His urine and semen specimens have all been negative for tubercle bacilli since this treatment. Eighteen twenty-four-hour cultures of the voided urine and two sets of ureteral cultures have been examined, at intervals, since treatment. The last examination was in March, 1953, a period of eighteen months since the cessation of treatment. The husband has continued asymptomatic throughout and since the treatment.

## COMMENT

It is apparent that venereal infection with tuberculosis is possible but is not frequent. The table compiled by Hellerstrom (1), to which the present case and the case of Obrant (2) have been added, demonstrates that vulvar tuberculosis in the wife resembles lymphogranuloma inguinale or primary syphilis. Indeed, the diseases may exist together. This close resemblance plus the rarity of the disease characteristically lead to a long delay in the diagnosis. It is interesting that the length of time between the coitus at which the wife is exposed and the development of the ulcer and inguinal lymphadenopathy may be as short as three or four weeks (1). In the present case, however, the infected semen had been ejaculated, without protection, into the wife's vagina on many occasions over a ten-year period before she too became infected.

The pathways of infection which led to the tuberculous involvement of the genitalia in the husband and the subsequent infection in his wife appeared to be the same as those in the majority of the male patients with genital tuberculosis studied at this Center for Genito-Urinary Tuberculosis. The men who developed a tuberculous epididymitis were usually found (like this man) to have an active focus in the prostate gland. Cultures of their semen were frequently positive for tubercle bacilli. It was presumed that the tubercle bacilli infected the epididymis by descending the ductus deferens from the prostate gland. The majority (85 per cent) of the men with tuberculosis of the prostate showed evidence of a tuberculous lesion in the kidneys, which presumably had infected the prostate. Tubercle bacilli which had been washed down by the urine had entered the prostatic ducts and established foci in their lumina. In addition, there was usually some evidence of an old pulmonary infection, calcified lymph nodes, or a history of contact with a known active source of pulmonary tuberculosis. Most of the renal tuberculosis at this center was secondary to pulmonary tuberculosis, even though the pulmonary lesion had often healed, leaving only the genitourinary foci active.

The fact that more husbands with prostatic tuberculosis do not infect their wives may be due to the resistance of the vaginal epithelium to infection by tubercle bacilli which are applied topically via the semen. It may also reflect the fact that prostatic tuberculosis gradually destroys the glandular elements of the prostate. As a result of this destruction, the volume of the semen decreases progressively. Eighty per cent of one group of the writers' patients with prostatic tuberculosis showed a semen volume under 1.5 cc., and 55 per cent had less than 0.5 cc. of semen. Many of the patients comment that orgasm produces only slight moisture at the tip of the penis, although a few moments later a drop of (prostatic?) fluid may exude from the meatus. Perhaps these greatly reduced quantities of semen rarely reach the vagina. Even then, the volume of tuberculous inoculum may frequently be too scanty to infect the vaginal or vulvar epithelium.

It is interesting, if irrelevant, to note that, of one group of forty married men with tuberculous genital infections, only one child had been produced since the development of tuberculosis in the prostate gland. Several of these men had had children previous to the development of the disease.


## SUMMARY

A rare case of infection of the wife by the husband's tuberculous semen has been reported. This case has been added to a tabulation of eight similar cases found in the European literature. In the present case it was demonstrated that the husband's semen and urine were positive for *M. tuberculosis* by culture. It appeared probable that the vulvar lesion caused in the wife represented a primary tuberculous infection. Surgical excision of the inguinal adenopathy in the wife was accomplished after token preoperative "preparation" with dihydrostreptomycin and was completely successful. Treatment of the husband's prostatic and possible renal tuberculosis was given in the form of daily streptomycin and para-aminosalicylic acid (PAS) for a period of one year. His urine and semen, which were both positive for *M. tuberculosis* at the beginning of therapy, have been sterile for a period of eighteen months.

It was only after excision and histopathologic diagnosis of the draining lymph nodes in the wife's groin that the retrospective discovery of genital tuberculosis in the husband was made. It was then apparent that the wife's vulva had been infected by the husband's semen.

## SUMARIO

*Transmisión de Tuberculosis Genital del Esposo a la Esposa por Vía del Semen*

Preséntase un caso raro de infección de la esposa por el semen tuberculoso del marido, agregándose este caso a una tabulación de 8 casos semejantes descubiertos en la literatura europea. En el caso actual, se demostró, por medio de cultivos, que el semen y la orina del esposo eran positivos para el *M. tuberculosis*. Parece probable que la lesión vulvar provocada en la mujer representase una infección (tuberculosa) primaria. Se llevó a cabo la excisión quirúrgica de la adenopatía inguinal en la esposa, después de la "preparación" preoperatoria tentativa con dihidroestreptomicina, con un éxito rotundo. El tratamiento de la tuberculosis prostática y posiblemente renal del marido fué administrado en forma diaria de estreptomicina y ácido para-aminosalicílico durante un período de un año. La orina y semen del mismo, que eran positivos al comenzar la terapéutica, han permanecido negativos por un período de dieciocho meses.

Sólo después de la excisión y del diagnóstico histopatológico de los ganglios linfáticos supurantes de la ingle de la esposa fué que tuvo lugar el descubrimiento retrospectivo de tuberculosis genital en el esposo. Resultó entonces evidente que la vulva de la mujer había sido infectada por el semen del marido.

RESUME

*Un cas de tuberculose vulvaire chez une femme dont le mari présente une tuberculose prostatique*

Un cas rare d'infection de la femme par le sperme tuberculeux du mari est rapporté. Ce cas s'ajoute à une liste de huit autres cas similaires découverts dans la littérature médicale européenne. Dans ce cas il a été démontré par la culture que le sperme et l'urine du mari étaient "positifs" pour le *M. tuberculosis*. La lésion vulvaire provoquée chez la femme paraît représenter une primo-infection (tuberculeuse). L'excision chirurgicale de l'adénopathie inguinale chez la femme a été effectuée après "préparation" diagnostique préopératoire par la dihydrostreptomycine et elle a été complètement satisfaisante. Chez le mari, le traitement de la tuberculose prostatique, et probablement aussi rénale, a consisté en administration quotidienne de streptomycine et d'acide para-aminosalicylique, pendant une période d'une année. L'urine et le sperme qui étaient tous deux "positifs" au commencement du traitement sont restés "négatifs" pendant une période de dix-huit mois.

Ce ne fut qu'après l'excision et le diagnostic histo-pathologique des ganglions lymphatiques inguinaux fistulisés que la découverte rétrospective d'une tuberculose génitale du mari fut faite. Il est alors devenu apparent que la tuberculisation de la vulve chez cette femme était imputable au sperme du mari.

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# ACUTE BENIGN HISTOPLASMOSIS[1]

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The literature contains few proved examples of the acute severe benign form of pulmonary histoplasmosis as it occurs in adults. The present paper describes such a case, recognized and treated in Seattle, Washington, in which the diagnosis was confirmed by the complement fixation test. The relation of this type of case to the disease as a whole will be considered.

## CASE REPORT

V. J., a 19-year-old Negro male, was admitted to the United States Army Hospital, Fort Lawton, Washington, on August 23, 1952, complaining of cough and malaise of one week's duration. Itching of the skin over the right wrist and upper back began one day before entry. He had previously been in good health, and had always lived on the outskirts of Winfield, Louisiana. He entered the Army in March, 1952, and took his basic training at Fort Bliss, Texas, from March to July, 1952. The area was dusty, and he was in foxholes often. He went to his home in Louisiana for a three-week furlough late in July. Chickens were kept in the backyard, but he had no close contact with them and did not enter the chicken coop. He then traveled by train directly to Seattle, by way of Kansas City and Portland. He arrived in Seattle on August 18, two days after the cough began.

On admission to the hospital he appeared acutely ill, with a temperature of 101°F., and was coughing spasmodically but producing very little sputum. Slight erythema and scaling were noted over the upper trunk. A few small vesicles were present on the uvula. Examination of the lungs was essentially negative except for the presence of a few high-pitched rales over both lung fields. There was marked tenderness in both upper quadrants of the abdomen, but neither the liver nor spleen seemed enlarged. No adenopathy was present. The leukocyte count was 12,500 per cu. mm., with 28 per cent polymorphonuclear leukocytes, 65 per cent lymphocytes, 4 per cent monocytes, and 3 per cent eosinophils. The erythrocyte sedimentation rate (Wintrobe method) was 13 mm. per hour. Results of urinalysis were normal, and blood cultures were sterile. Routine sputum culture revealed the usual nasopharyngeal flora, and cultures for fungi were negative. Seven concentrated sputum specimens were negative for tubercle bacilli on microscopic examination and culture. Blood smears for malarial parasites were negative. *Brucella* and tularemia agglutinations were negative, and the test for heterophile antibodies varied from negative to 1 to 14 during the first month of illness. Roentgenogram of the chest on August 24 revealed many small (2 to 6 mm.) patchy densities scattered evenly throughout both lung fields, and there was moderate hilar adenopathy. A film obtained four days later was unchanged and is reproduced in figure 1.

The Mantoux test (second-strength PPD) was positive during the third week of illness. The skin test with histoplasmin (1:100) was performed six weeks after onset and was strongly positive. A skin test with coccidioidin (1:100) performed at the same time was negative. Complement fixation test for histoplasmosis seven weeks after onset (October 2, 1952) was positive in a dilution of 1:64, and thirteen weeks after onset (November 16, 1952) was positive in a dilution of 1:512. These tests were performed at the Communicable Disease Center, Atlanta, Georgia.

The patient was acutely ill during the first week in the hospital (figure 2). There was a

[1] From the X-ray Department, United States Army Hospital, Fort Lawton; and Department of Medicine, University of Washington School of Medicine, Seattle, Washington.

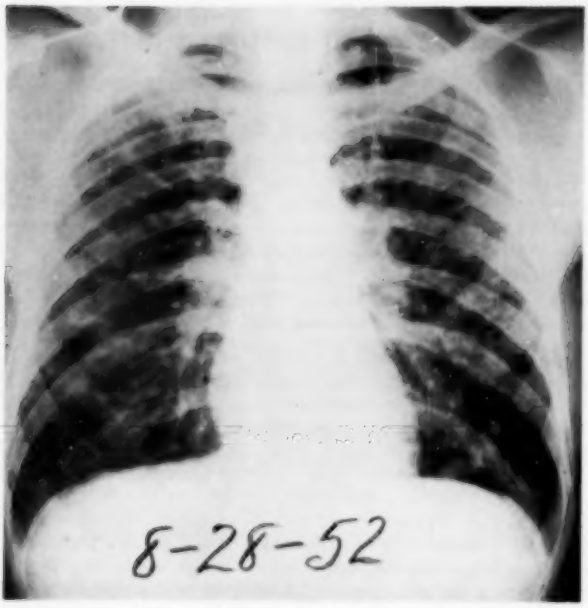


FIG. 1. Roentgenogram of chest taken early in the course of the illness, showing multiple infiltrations throughout the lung fields and enlargement of hilar lymph nodes.

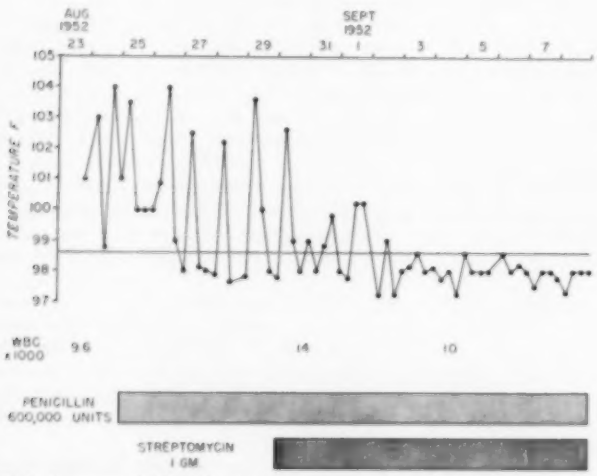


FIG. 2. Clinical course during the first two weeks of hospitalization. The patient was quite ill, and the spiking temperature was associated with shaking chills.

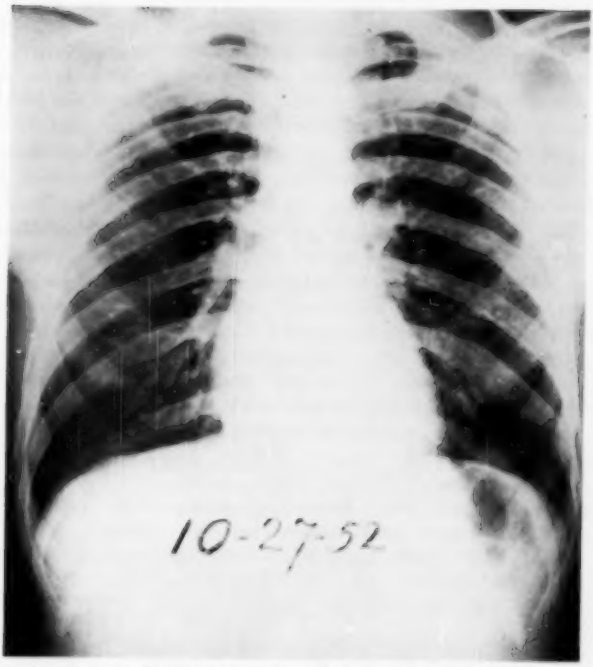


FIG. 3. Roentgenogram of chest two and one-half months after onset, showing marked, but incomplete, clearing of pulmonary infiltrations. Hilar adenopathy is no longer present.

spiking fever, and severe shaking chills occurred on several occasions. The course was probably unaffected by antimicrobial therapy, although the temperature returned to normal abruptly after streptomycin was started. Malaise and headache were marked, and the severe hacking cough, productive of only small amounts of mucoid sputum, persisted until the temperature declined to normal. Thereafter he regained his strength rather slowly and was essentially asymptomatic within six to eight weeks. Repeated blood examinations revealed a definite lymphocytosis, which persisted for approximately eight weeks. A representative count during the fifth week of illness revealed a leukocyte count of 6,900 per cu. mm., with 44 per cent polymorphonuclear leukocytes, 51 per cent lymphocytes, 2 per cent monocytes, and 3 per cent eosinophils. The highest erythrocyte sedimentation rate recorded was 28 mm. per hour (Wintrobe method) during the fifth week of illness, and within the next three weeks it had returned to normal. Repeated roentgenograms of the chest showed little change during the first three or four weeks, but thereafter the hilar adenopathy and pulmonary infiltrations gradually receded. However, small, more fibrotic-appearing infiltrations were still present on the film taken two and one-half months after entry (figure 3). The patient was returned to full military duty on December 3, 1952.

## COMMENT

Ten years ago histoplasmosis was regarded as a generalized, almost uniformly fatal infection (1). Since then, as a result of extensive skin testing and roentgenographic surveys, it has become apparent that an extremely mild, asymptomatic form of the disease occurs commonly in certain central and southern states and often leads to multiple pul-

monary calcifications (2, 3). Between these two extremes a form of the disease occurs, chiefly in adults, in which there is a severe febrile illness of approximately two weeks' duration, associated with multiple infiltrations throughout the lung fields and with hilar adenopathy. A number of epidemics now known to be examples of this form of the disease have been reported in the last decade, but in none was the diagnosis definitely established at the time. In all of these outbreaks the patients had had heavy exposure to dust contaminated with excreta of rodents, chickens, or pigeons. Schwartz and Spitz described a six-year follow-up of one of the soldiers who was seriously ill during the Camp Gruber outbreak in 1944 (4). Multiple calcifications had developed throughout the lung fields, the histoplasmin skin test was positive, and the complement fixation test was positive in a dilution of 1:140. These suggestive findings have been confirmed by the recent comprehensive report of Furcolow and Grayston (5) which provides convincing evidence that more than a dozen such outbreaks were actually instances of a severe but benign form of pulmonary histoplasmosis. *Histoplasma capsulatum* was isolated from soil samples in 11 of 13 epidemics, and skin tests and serologic studies indicated that the patients had been infected with this organism. Pulmonary calcification was demonstrated in 61 of 73 persons, and in 42 the process was the disseminated miliary type. Many of the 116 patients were seriously ill, but there was only one death. This occurred in a five-month-old child whose disease progressed to the disseminated form.

The severe but benign pulmonary form of the disease occurs in single cases as well as in epidemics, as is illustrated by the present case report. There was no definite indication where this patient contracted the disease. Infectious material could have been inhaled during the three-week furlough at his home in Louisiana, or a chance exposure could have occurred as he passed through Arkansas, Oklahoma, and Kansas by train on the way to Seattle. In the epidemics reported, when the exact time of exposure was known, the incubation period varied from four to fifteen days. Onset of the illness in Seattle emphasizes the fact, now well recognized in relation to coccidioidomycosis, that with a transient population this type of disease is likely to be encountered outside endemic areas.

It is rarely possible to isolate the fungus in this form of the disease, and the diagnosis must therefore be confirmed serologically. Schwartz and Spitz noted that, in most of the epidemics reported in the literature, complement fixation tests were performed too early during the convalescent period to be conclusive. Another important point, emphasized by Furcolow and Grayston (5), is that serologic techniques have been greatly improved in the last five years. In the present case, a rising titer was observed quite late, being 1:64 at seven weeks and 1:512 thirteen weeks after onset. These are relatively high titers, possibly because of the severity of the infection. Recent studies of the complement fixation test indicate that considerably lower values may be associated with active infection when the involvement is less extensive (6, 7). The complement fixation test would appear to be most useful between the fourth and twentieth weeks following infection because of the slow rise and late fall in antibody titer (5, 6, 7).

The appearance of the chest roentgenogram in the present case is similar to that encountered in roentgenographic surveys in schoolchildren in Kansas City (3). In contrast to the acute illness which occurred in the present patient and in the epidemics cited, these children were entirely asymptomatic and remained in school. Whether the difference in response is related to age, to the size of the infecting dose, or to previous contact with the fungus cannot be stated at present.

In contrast to the widespread pulmonary infiltrations observed in the present case and in the various epidemics, it should be noted that a single infiltrate has been de-

scribed in some cases of benign histoplasmosis. This situation has been well summarized by Beadenkopf and Loosli (8), and cases of this sort have recently been described in laboratory workers by Furcolow and co-workers (7).

SUMMARY

A case of acute benign pulmonary histoplasmosis has been presented. The clinical and laboratory features included a severe febrile illness of approximately two weeks' duration, severe spasmodic coughing with little sputum, shaking chills, itching skin with erythematous rash, normal or only slightly elevated total leukocyte count, relative lymphocytosis persisting for several weeks, and multiple pulmonary infiltrations and hilar adenopathy, clearing slowly and incompletely. Sufficient time has not elapsed for calcifications to develop. The diagnosis was confirmed by performing serologic studies relatively late during convalescence. During the seventh week after onset, the complement fixation test was positive in a dilution of 1:64 and, during the thirteenth week, in a dilution of 1:512.

SUMARIO

*Histoplasmosis Benigna Aguda*

El caso presentado es de histoplasmosis pulmonary benigna aguda. Las características clínicas y de laboratorio comprendieron una enfermedad febril grave de unas dos semanas de duración, intensa tos espasmódica con poco esputo, escalofríos agitantes, prurito cutáneo con erupción eritematosa, leucocitosis total normal o apenas elevada, linfocitosis relativa que persistió por varias semanas y múltiples infiltraciones pulmonares y adenopatía hiliar, que desaparecieron lenta e incompletamente. No ha transcurrido suficiente tiempo para que se presenten calcificaciones. El diagnóstico se confirmó ejecutando estudios serológicos relativamente tarde durante la convalecencia, Durante la séptima semana consecutiva a la iniciación, la prueba de fijación del complemento resultó positiva a una dilución de 1:64 y, durante la décimatercera semana a una dilución de 1:512.

RESUME

*Histoplasmose bénigene aiguë*

Présentation d'un cas d'histoplasmose bénigne aiguë. Les caractères cliniques et les examens de laboratoire consistaient en une maladie fébrile sévère d'environ trois semaines, une toux spasmodique intense avec peu de sécrétions, de grands frissons, un érythème cutané avec prurit, une numération normale ou légèrement élevée de la totalité des leucocytes, une lymphocytose relative persistant pendant plusieurs semaines ainsi qu'un infiltrat pulmonaire avec adénopathie hilaire, qui s'est éclairci lentement et incomplètement. La durée de l'affection a été trop brève pour que la calcification des lésions se manifeste. Le diagnostic a été confirmé par des études sérologiques, qui ont été effectuées relativement tard au cours de la convalescence. Pendant la septième semaine qui a suivi le début de la maladie, le test de fixation du complément était positif à la dilution de 1:64 et, pendant la treizième semaine à la dilution de 1:512.

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(2) Christie, A., and Peterson, J. C.: Pulmonary calcification in negative reactors to tuberculin, Am. J. Pub. Health, 1945, *35*, 1131.

(3) Furcolow, M. L.: Development of calcification in pulmonary lesions associated with sensitivity to histoplasmin, Pub. Health Rep., 1949, *64*, 1363.

(4) Schwartz, B., and Spitz, L. J.: Histoplasmosis in epidemic form, A. M. A. Arch. Int. Med., 1952, *89*, 541.
(5) Furcolow, M. L., and Grayston, J. T.: Occurrence of histoplasmosis in epidemics: Etiologic studies, Am. Rev. Tubere., 1953, *68*, 307.
(6) Grayston, J. T.: A study of the complement fixation reaction in histoplasmosis, J. Lab. & Clin. Med., 1952, *40*, 90.
(7) Furcolow, M. L., Guntheroth, W. G., and Willis, M. J.: The frequency of laboratory infections with *Histoplasma capsulatum*: Their clinical and X-ray characteristics, J. Lab. & Clin. Med., 1952, *40*, 182.
(8) Beadenkopf, W. G., and Loosli, C. G.: Histoplasmosis, tuberculosis and coccidioidomycosis, J. A. M. A., 1951, *146*, 621.

# *Editorial*

## The Fiftieth Anniversary of the National Tuberculosis Association

Fifty years have passed since a group of physicians and other citizens interested in the control of tuberculosis met in a schoolhouse in Atlantic City and founded a voluntary organization, the National Association for the Study and Prevention of Tuberculosis, to fight this disease. At that time tuberculosis was the chief cause of death in the United States, killing more than one hundred and fifty thousand persons annually and reducing to ineffectiveness each year half a million more, most of whom were ultimately to die from the illness. Among the most ardent founders were such noted physicians as Edward L. Trudeau of Saranac Lake, Lawrence F. Flick of Philadelphia, William H. Welch and William Osler of Baltimore, Hermann M. Biggs of New York, Theobald Smith and Vincent Y. Bowditch of Boston, and General George M. Sternberg of Washington. The total list is a long one, including scores of other eminent doctors and welfare workers.

Thirteen years later a group of research-minded leaders in the young and vigorous association, recognizing the need for a technical journal to broaden knowledge and stimulate scientific investigation on tuberculosis in this country, started THE AMERICAN REVIEW OF TUBERCULOSIS. The aims of the new journal were set forth in an editorial in the first issue, written by the first managing editor, Allen K. Krause, who was to dominate American thought on the pathogenesis of tuberculosis for a decade to come. The REVIEW was in many respects an outgrowth of the Saranac Lake tuberculosis school. Trudeau, who had died but two years before the REVIEW was commenced, had trained most of the men who founded the journal. His successor as head of the Saranac Laboratory, Edward R. Baldwin, was named editor-in-chief and Krause the managing editor. The distinguished Lawrason Brown, who was for many years the guiding spirit of the Trudeau Sanatorium, was an early and frequent contributor.

The first issue was prophetic in its vista of tuberculosis problems to be met and solved. The opening article was on the classification of pulmonary tuberculosis, long since recognized as a perennial task. The second number opened with Krause's "Nature of Resistance to Tuberculosis," an American classic in phthisiology, which crystallized the best thinking of the day on immunological aspects of the disease. It was a time when one could speak, as did Krause himself in the REVIEW's first editorial, of the "universality of first infection," and it was not too early to acknowledge that "in the last analysis the fight against tuberculosis must be along broader lines than those which are purely medical," i.e., with full recognition of the important role of social and economic forces.

The first volume of the REVIEW carried articles on roentgenographic diagnosis, pneumothorax, chemotherapy, surgery, and public health aspects of the disease. Roentgenography was new, films had not yet replaced glass plates,

"Dunham's fan" was a focal point in the X-ray picturization of the disease, the relative merits of physical diagnosis and X-ray photography were debated, and wise clinicians like Louis Hamman stressed the role of each. It was war time too, and the great importance of tuberculosis as a military problem was pointed out by the Army's chief medical officer in this specialty, Colonel George E. Bushnell, in an article that set the stage for a fight by the Armed Forces to control the disease. In passing, it may be noted that unknowingly Colonel Bushnell forecast issues to be met a quarter of a century later and again reflected in the columns of the REVIEW.

Sixty-seven volumes have followed the first one. A volume of the REVIEW now consists of six rather than twelve monthly issues, and the total annual paging is more than twice as great as in the early years. The latest volume has been concerned in large measure with the clinical and laboratory aspects of antimicrobial therapy, a science in its infancy in 1917, and yet represented by several significant articles in the opening numbers. One field represented in the final issue of 1953, pulmonary resection, was quite unanticipated in 1917.

In the intervening years, more than four thousand original articles have appeared. They have covered all phases of the investigation of tuberculosis and have included a number of famous sequences in which knowledge grew rapidly from the labors of a single investigator or the pooled discoveries of several, e.g., on allergy and immunity, the nature of tuberculin and the tuberculin reaction, the value and defects of artificial pneumothorax, chemotherapy, pulmonary fibrosis due to mineral dusts, BCG vaccination, racial variations in tuberculosis, and mass case finding in tuberculosis control.

In these and scores of other fields, contributors to THE AMERICAN REVIEW OF TUBERCULOSIS have improved our understanding of this disease and stimulated hundreds of able investigators to further productive effort. Since 1940 the REVIEW has been the official journal of the American Trudeau Society, the medical section of the National Tuberculosis Association. Through its columns the five thousand members of the society are united in a solidarity of effort unattainable without such a medium.

In the half century since the founding of the National Association on June 6, 1904, knowledge of tuberculosis has increased in every aspect, methods of medical and surgical treatment have improved enormously, and highly effective public health measures for its control have been established. Through the persistent effort and constant support of the National Association and its affiliates, federal and local organizations for control of tuberculosis as a public health problem have been widely developed, with corresponding effect in suppression of the disease. The death rate from tuberculosis has dropped to less than one-tenth of that prevailing in 1904, and prospects are bright for its continuing rapid reduction. Nevertheless, the fight is far from won, and the very measures that have led to success in the campaign cost hundreds of millions of dollars annually. Leaders of the Association emphasize strongly that the time has not yet come for relaxation of effort. Rather, it is a ripe period to intensify measures for control, when organization for the campaign is so effective.

Realistically, however, foreseeing the ultimate, if still distant, eradication of tuberculosis in this country, successive Boards of Directors in recent years have given much thought to the future of the National Tuberculosis Association's remarkable and powerful machinery for education, medical research, and public health promotion. Some change in direction is inevitable as the campaign against tuberculosis approaches its final objectives. Already certain types of expanded program are authorized for the state affiliates of the national body, and wider latitude may be anticipated in the years immediately ahead.

The future of the medical section of the Association, the American Trudeau Society, and that of its official journal, THE AMERICAN REVIEW OF TUBERCULOSIS, are equally at issue. The Trudeau Society, however, has for years recognized fundamental relationships in the study of tuberculosis and other chronic diseases of the lungs. A wide overlap occurs in diagnosis, physiological techniques of investigation, surgical treatment, and rehabilitation. Expansion into the field of chronic pulmonary disease, and from that to other diseases of the lungs, seems not only natural but inevitable. In full accord with this view, the membership of the Trudeau Society, on recommendation of the Editorial Board of the REVIEW, recently added the subtitle *A Journal of Pulmonary Diseases* to the long-established official name of the periodical. At this time it would seem neither necessary nor wise, however, to steer the REVIEW with conscious effort into the newly opened channels. A natural development in this respect may be expected. It is perhaps worth while to point out that the expansion is not unprecedented. The second article of the first issue of the REVIEW, in March, 1917, dealt specifically and exclusively with "chronic-nontuberculous lung infection," outlining at length the principles and treatment of bronchiectasis, and many papers on other nontuberculous diseases of the lung have appeared in the pages of the REVIEW since then.

In its extended domain, as well as that long established, THE AMERICAN REVIEW OF TUBERCULOSIS will continue to render full service to the National Tuberculosis Association as it moves forward in its second fifty years. The enthusiasm of the members of the medical section of the Association is undiminished, and the REVIEW will have a great part to play in advancing knowledge in the widened field of pulmonary disease.

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# *Notes*

## AN APPRAISAL OF THE THEORIES OF PLEURITIC PAIN

There are two distinctive features of pleuritic pain which require particular attention. The characteristic pain increases in severity during inspiration, while on expiration the pain gradually subsides. The pain, moreover, is strictly confined to the lower part of the chest. The present writer attempted an appraisal of the four principal theories dealing with the factors underlying the production of this unique pain.

*The Friction Theory*, the oldest and most widely accepted, attributes the pain to friction which is produced by the apposition and movement of the two roughened pleural surfaces. There are a number of objections to this theory, the principal one being that pain is present after friction sounds can no longer be detected. This point can be readily demonstrated by directing the patient to hold the breath at the height of inspiration. This procedure immobilizes the pleurae and eliminates friction, yet the maximum degree of pain is experienced at this moment and continues until relieved by the succeeding expiration. It seems justifiable, therefore, to assume from this single illustration that friction is not an important element in the production of pleuritic pain.

*The Muscle Theory* of Sir James MacKenzie.[1] He states: "In a great number of cases when the ribs have been resected I have repeatedly explored the pleural cavity for any evidence of sensation and I could employ no form of stimulation capable of producing pain." After coming to the decision that the pleurae were insensitive, he ascribed the pain to contraction of hyperalgesic intercostal muscles. It is true that the pleura may become insensitive when the inflammation is of long standing, but later investigations present evidence that the parietal pleura is sensitive, the visceral insensitive, to pain.

*The Traction Theory* more recently advocated by Meakins[2] places the seat of the pain in the tissues immediately underlying the parietal pleura. "The distribution of pleural pain is along the course of the intercostal nerves, and is caused during respiration or coughing by the roughened visceral pleura through traction stretching the sensitive nerves immediately beneath the parietal pleura."

In lobar pneumonia the inspiratory pain may occur within a few hours after the onset of the illness and before the friction sounds of pleurisy are demonstrable. The state of the pleura during these initial hours is unknown. However, when extensions of the disease occurred in the opposite healthy lung within twelve hours of death, the present writer found that the pleura was acutely inflamed but was not roughened, as determined by inspection and palpation. Even when the disease terminates fatally on the third or fourth day, the pleura over the consolidated lobe, although dull in appearance, is not noticeably roughened and the flecks of exudate which may be present are easily removed. One is inclined, from the clinical and pathologic evidence at hand, to accept the traction theory with reserve.

*The Tension Theory.*[3] The smooth surfaces of the pleura are provided with a lubricant. They retain their smoothness during respiration by means of a rich supply of elastic fibers. The pleura is a perfectly designed mechanism for the reduction of friction incident

[1] MacKenzie, J.: Symptoms and Their Interpretation, ed. 4, Shaw & Son, London, 1920, p. 221.

[2] Meakins, J. C.: Practice of Medicine, ed. 4, The C. V. Mosby Company, St Louis, 1935, p. 484.

[3] Bray, H. A.: Am. Rev. Tubere., 1926, *13*, 14.

to respiration. Dogiel[4] identified sensory end organs in the parietal pleura of man. King[5] confirmed this observation and also found similar structures in the parietal pleura of the calf, the rabbit, the guinea pig, and the rat. The sensitiveness of the parietal pleura and the insensitiveness of the visceral pleura have been established to the satisfaction of most observers by the study of Capps[6] and by more recent observations following the advent of thoracic surgery.

According to the observations of the present writer, the pain is initiated as the chest expands and exerts tension on the sensitive, inflamed parietal pleura. As the tension is increased, the pain mounts in intensity. At the end of inspiration the tension on the pleura is at its height and the maximum degree of pain is experienced. The succeeding expiration reduces the tension, and the pain gradually subsides.

The localization of the pain to the lower part of the chest is due apparently to the considerably greater expansion of the chest in this region and the consequent greater tension on the parietal pleura in this area.

The factors responsible for the pain became more apparent in a group of tuberculous patients who developed an acute pleurisy with the characteristic inspiratory pain after the pleural surfaces had been widely separated by therapeutic pneumothorax. In this group of patients, friction and traction can be reasonably excluded as causal agents of the pain. The writer further noted in these patients that the pain, as in simple pleurisy, was alleviated, or completely disappeared, with the advent of an effusion.

*In conclusion:* According to the tension theory, the localization and the characteristic accentuation of the pain on inspiration and the subsidence of the pain on expiration are closely related to the degree of tension exerted on the sensitive and inflamed parietal pleura. During the expansion of the chest the tension on the pleura is steadily increased, and concurrently the pain mounts in intensity. At the end of inspiration the tension is at its height and the maximum pain is experienced. Conversely, as the chest contracts, the tension is gradually reduced and the pain subsides.

It seems reasonable to assume that the pain is confined to the lower part of the chest because in this region the expansion and consequent tension on the pleura is most marked.

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August 3, 1953

[4] DOGIEL, A. S.: Arch. f. mikr. Anat., 1903, *62*, p. 244.
[5] KING, M. J.: Ray Brook Sanatorium, Ray Brook, N. Y., unpublished report, 1925.
[6] CAPPS, J. S.: Arch. Int. Med., 1911, *8*, 717.

## METHODS OF DRAINAGE FOLLOWING PULMONARY RESECTION

Realizing that there is considerable variation in methods and techniques used by different surgeons for drainage following pulmonary resection, and being uncertain whether the present writers were using the most satisfactory methods, it was decided to survey the drainage methods being used by a number of qualified thoracic surgeons. A simple questionnaire was devised and sent to surgeons whose procedures were believed to be representative of current practices in this country. When a number of surgeons were associated in their institutions or practices, questionnaires were sent to only one member of the group with the request that, if a different method was used by any of his associates, the senders be so advised so that additional questionnaires could be filled out. In two instances, this was necessary. Since methods apparently do not vary in the other associated groups, the replies received from 64 surgeons[1] undoubtedly indicate the methods used by far greater numbers of surgeons.

The first question asked was in regard to the use of underwater drainage following pulmonary resections of lesser extent than total pneumonectomies. The answers were as follows:

| | |
|---|---|
| Underwater drainage without routine suction | 40 |
| Underwater drainage with routine suction | 22 |
| No drainage bottle routinely | 2 |

[1] Barkley, Howard T., Houston, Texas; Blades, Brian, Washington, D. C.; Brantigan, Otto C., Baltimore, Maryland; Brewer, Lyman A., Los Angeles, California; Budgen, Walter F., Syracuse, New York; Burford, Thomas H., St. Louis, Missouri; Byron, Francis R., Los Angeles, California; Carr, Duane, Memphis, Tennessee; Chamberlain, John Maxwell, New York, New York; Clagett, O. Theron, Rochester, Minnesota; Coleman, Frank Philip, Richmond, Virginia; Cotton, Bert Hollis, Beverly Hills, California; Curreri, Anthony R., Madison, Wisconsin; Davis, Edgar W., Washington, D. C.; Dodrill, Forest D., Detroit, Michigan; Evans, Byron H., Fresno, California; Gebauer, Paul, Honolulu, T. H.; Glover, Robert P., Philadelphia, Pennsylvania; Goldman, Alfred, Beverly Hills, California; Grow, John B., Denver, Colorado; Haight, Cameron, Ann Arbor, Michigan; Harter, John S., Louisville, Kentucky; Hausmann, Paul F., Milwaukee, Wisconsin; Heroy, William W., Huntington, New York; Hollingsworth, Russell K., Martinsburg, West Virginia; Hughes, Felix A., Jr., Memphis, Tennessee; Jarvis, Fred J., Seattle, Washington; Jensen, Nathan K., Minneapolis, Minnesota; Jones, John C., Los Angeles, California; Kay, Earle B., Cleveland, Ohio; Kelley, Winfield O., Norwich, Connecticut; Kinsella, Thomas J., Minneapolis, Minnesota; Langston, Hiram T., Chicago, Illinois; Laurey, James R., Washington, D. C.; Lees, William M., Chicago, Illinois; Maier, Herbert C., New York, New York; Major, Robert C., Augusta, Georgia; Meade, Richard H., Grand Rapids, Michigan; Melick, D. W., Phoenix, Arizona; Moore, Julian A., Asheville, North Carolina; Murphy, James D., Oteen, North Carolina; O'Rourke, Paul V., Detroit, Michigan; Overholt, Richard H., Boston, Massachusetts; Raine, Forrester, Milwaukee, Wisconsin; Ryan, Bernard J., Bay Shore, New York; Samson, Paul C., Oakland, California; Sanger, Paul W., Charlotte, North Carolina; Seybold, William D., Houston, Texas; Shaw, Robert R., Dallas, Texas; Shefts, Laurence M., San Antonio, Texas; Sloan, Herbert F., Ann Arbor, Michigan; Sommer, George N. J., Jr., Trenton, New Jersey; Strieder, John W., Boston, Massachusetts; Stringer, C. J., Lansing, Michigan; Van Hazel, Willard, Chicago, Illinois; Weinberg, Joseph A., Long Beach, California; Weisel, Wilson, Milwaukee, Wisconsin; White, M. Lawrence, Jr., Huntington, West Virginia; Wilson, Norman J., Boston, Massachusetts; Wiper, Thomas B., San Francisco, California; Woodruff, Warriner, Saranac Lake, New York; Woods, Francis M., Boston, Massachusetts; Young, William P., Madison, Wisconsin.

The second question asked the amount of negative pressure used if suction were used even occasionally. The following answers are tabulated in centimeters of water, although a few used mercury regulators:

| | |
|---|---|
| 0–5 cm. 4 | 10–20 cm. 14 |
| 5–10 cm. 12 | 20–30 cm. 5 |
| 10–15 cm. 14 | 30–50 cm. 1 |
| 15–20 cm. 1 | 120–150 cm. 1 |

The various sources of suction were as follows:

| | |
|---|---|
| Stedman pump | 17 |
| Wall source | 15 |
| Unspecified motor pump | 12 |
| Chamberlin-Daniels pump | 9 |
| Gomco thermatic pump | 8 |
| Water faucet | 5 |
| Chafin-Pratt pump | 4 |
| Miscellaneous other sources | 6 |

The next question was what types of drainage tubes are used. Some surgeons use more than one kind. All kinds mentioned were tabulated:

| | |
|---|---|
| Catheters with multiple holes | 27 |
| Pezzar catheters | 11 |
| Rectal tubes | 9 |
| Foley catheters | 9 |
| Right angle catheters | 4 |
| Latex tubes | 3 |
| Plastic tubes | 2 |
| Miscellaneous | 5 |

The number of drainage tubes sometimes varied according to individual circumstances. For the most part, the routine use of a certain number was indicated. All variations were tabulated:

| | | | |
|---|---|---|---|
| One tube | 18 | Three tubes | 4 |
| Two tubes | 46 | Four tubes | 1 |

The final question asked whether drainage was used for total pneumonectomies.

| | |
|---|---|
| No Routine Drainage | 49 |
| *Exceptions:* | |
| *Drainage when pneumonectomy is done under thoracoplasty* | 14 |
| *Drainage when bronchial leak is feared* | 5 |
| Routine Drainage | 15 |
| *Methods:* | |
| *Underwater drainage routinely* | 6 |
| *Tube inserted for measuring pressure* | 8 |
| *Method not specified* | 1 |

DEPARTMENT OF SURGERY
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October 27, 1953

## TUBERCULOSIS MORTALITY RATES AMONG WORLD WAR II VETERANS (A SCREENED POPULATION) FOR THE YEARS 1948 THROUGH 1952

Since the beginning of World War II, there was a steady rise in the mortality rate from tuberculosis in the group of men accepted for service into the Armed Forces. This group, referred to as "one of peculiar significance," for it is a screened population from which the majority, if not all, of tuberculous cases were removed, had an average tuberculosis mortality of less than 4 per 100,000 in 1942; the mortality increased to 6 in 1943, 10 in 1944, and 12 in 1945, while the mortality rate for the civilian unscreened population, corrected for age, during the years under consideration remained at approximately 52 per 100,000.[1]

The mortality rate for 1946 and 1947 is not available at the present time. It is not possible to estimate the number of veterans who died of tuberculosis outside Veterans Administration hospitals for these two years.

Since 1948, after a Central Tuberculosis Register for the Veterans Administration was initiated, the available data permitted various estimates of morbidity and mortality rates among veterans for different regions of the country for any given year beginning with 1948.

The Veterans Administration Central Tuberculosis Register was initiated by: (*1*) filing concise summaries of the status of all World War II veterans discharged for tuberculosis by the Army, Navy, and Marine Corps since 1942, and (*2*) assembling of data from the essential basic documents prepared by the Veterans Administration, such as rating sheets (VA Form 8-564) and the Tuberculosis Case Reports (VA Form 10-2530).

The Tuberculosis Case Reports are received from all Veterans Administration hospitals, all regional offices, and Veterans Administration fee-basis physicians on all tuberculous veterans under their care. The rating sheets are received directly from adjudication services in the regional offices. The Central Register is current and contains presumably complete data on all World War II veterans (enlisted personnel and officers). In addition, a file is maintained on veterans (officers and enlisted personnel) with tuberculosis who served since June 27, 1950, in the Korean War. A suitable file is also maintained on veterans who have died of tuberculosis since 1947.

Analysis of the records thus assembled indicate that the estimated average annual tuberculosis mortality rate among World War II veterans for the period from 1948 through 1952 was 8.3 per 100,000. The estimated rates for each year were:

1948—10.8 per 100,000
1949— 8.4 per 100,000
1950— 7.9 per 100,000
1951— 8.0 per 100,000
1952— 6.2 per 100,000

In computing the tuberculosis mortality rate, all deaths which occurred outside the Veterans Administration hospitals among World War II veterans known to have tuberculosis were assumed to be tuberculous deaths. This was done since it was not possible to determine the cause of death of those persons and the nontuberculosis deaths in the group were not considered to be so numerous as to seriously affect the estimate. Additionally, there may have been veterans who died of tuberculosis who had never sought medical or financial assistance from the Veterans Administration and were therefore not included in the estimate. This group was assumed to be very small.

[1] Long, E. R.: Editorial, Am. Rev. Tubere., 1946, *54*, 319.

Beginning with the year 1948, several factors in the Tuberculosis Control Program of the Veterans Administration brought about the retarding influences of which Dr. Esmond R. Long spoke so prophetically in 1946,[1] such as (*1*) the new methods of treatment for tuberculosis (introduction of chemotherapy in 1946) and (*2*) the extensive Case-Finding Survey Program with its full impact in 1949 in all Veterans Administration hospitals and outpatient clinics. These factors, plus the decline of tuberculosis mortality in the general population, not only stopped the upward climb of the mortality trend among veterans, but brought a considerable drop (approximately 40 per cent) in the number of tuberculosis deaths among World War II veterans.

The effects of antimicrobial drugs in the treatment of tuberculosis as well as other improvements in treatment employed by the Veterans Administration are evidenced by the drop in the number of tuberculosis deaths in Veterans hospitals among World War II veterans from 990 in 1948 to 592 in 1952. During this period there was a slight drop in the number of World War II veterans who received treatment for tuberculosis in Veterans Administration hospitals, but that decrease was small in relation to the 40 per cent drop in the number of deaths from tuberculosis. Provisional data indicate that there will be a further decline during 1953 in the number of deaths from tuberculosis of World War II veterans in Veterans Administration hospitals.

The effect of the Case-Finding Program, while not as dramatic as the results of improved treatment methods, has, nevertheless, also been instrumental in lowering the tuberculosis mortality rate.

U. S. Veterans Administration
Department of Medicine and Surgery
Tuberculosis Control and Reports & Statistics Service
Washington 25, D. C.

Leo V. Schneider
Dan I. Rosen

December 10, 1953

# *Correspondence*

## VIRULENCE OF ISONIAZID-RESISTANT TUBERCLE BACILLI

*To the Editor of the American Review of Tuberculosis:*

The three recent publications (Peizer, L. R., Widelock, D., and Klein, S.: Am. Rev. Tuberc., 1953, *68*, 290; Steenken, W., and Wolinsky, E.: Am. Rev. Tuberc., 1953, *68*, 548; Bloch, H., Widelock, D., and Peizer, L. R.: Am. Rev. Tuberc., 1953, *68*, 734) which have appeared in your journal leave the impression that the correlation between the virulence of tubercle bacilli and their degree of resistance to isoniazid is in doubt. It is probable that some of the resistant strains employed in all three studies were composed of mixtures of bacilli with different degrees of resistance since, even in sensitivity tests where the amount of growth appears to be equal on slopes with and without the drug, Colwell, Pitner, and Moravec (Am. Rev. Tuberc., 1951, *63*, 679) have pointed out that the usual heavy inoculation of these slopes can mask the presence of a mixture of bacilli with different degrees of resistance. These investigations have established the important point that strains even highly resistant to isoniazid may contain a proportion of virulent bacilli. There is, however, no proof that these virulent bacilli compose the whole strain, nor that they are indeed resistant, nor that their presence bears any relation to the apparent degree of resistance of the strain. They may well be sensitive or less resistant than the remainder of the strain.

In order to establish a correlation between virulence and degree of resistance, it is thus necessary to be certain that the strains employed are entirely composed of bacilli with about the same degree of resistance. This was achieved by subcultivating strains on slopes containing a concentration of isoniazid a little lower than the minimal inhibitory concentration. Thirteen resistant strains from 12 patients were treated in this way, and 0.1 mg. of each strain was then injected intramuscularly into guinea pigs (Barnet, M., Bushby, S. R. M., and Mitchison, D. A.: Lancet, 1953, *1*, 314; Brit. J. Exper. Path., 1953, *34*, 568; Mitchison, D. A.: Brit. M. J., 1954, No. 4853, 128). The results of these virulence tests are summarized below. They suggest a very definite relation between virulence and degree of resistance.

| DEGREE OF ISONIAZID* RESISTANCE | GROWTH ON 0.2 γ PER ML. NOT ON 1 | GROWTH ON 1 γ PER ML. NOT ON 5 | GROWTH ON 10 γ PER ML. NOT ON 50 | GROWTH ON 50 γ PER ML. |
|---|---|---|---|---|
| Isoniazid concentration during subculture γ per ml. | 0 or 0.2 | 0.2 | 1 or 10 | 50 |
| Virulence in guinea pigs: | | | | |
| Full | 3 | 2 | 0 | 0 |
| Intermediate | 0 | 1 | 1 | 0 |
| Low | 0 | 1 | 2 | 7† |

* Measured on Löwenstein-Jensen slopes by the Medical Research Council (Lancet, 1953, *2*, 213) method.

† Three of these strains were variants of H37Rv, one having been obtained from a guinea pig infected with H37Rv and treated with isoniazid.

Strains of low virulence produced macroscopic lesions only at the site of inoculation and in the draining lymph glands. Strains of intermediate virulence produced macroscopic

disease in the spleen and other organs which tended to heal spontaneously. Both types of strain were of lowered virulence in mice when injected by the intravenous route. The virulence of the pretreatment sensitive strains corresponding to 10 of the 13 resistant strains from patients was tested; all were fully virulent. A good correlation between virulence and degree of resistance is apparent.

It will be noted that strains capable of growth on 1 $\gamma$ of isoniazid per ml. but not on 5 $\gamma$ per ml. were often fully virulent and could also cause experimental tuberculosis which was not affected by treatment with isoniazid at a dosage level higher than that used in man. Bacilli with this degree of resistance in "crude" resistant strains from patients may well account for their virulence, although they may also be due, in part, to bacilli of lower resistance or to persisting sensitive bacilli.

If the results of these virulence tests in animals can be applied to man, they imply that a proportion of isoniazid-resistant bacilli may be of lowered virulence in patients. However, the existence of fully virulent strains which can probably grow in the lesions of treated patients emphasizes the importance of using efficient combined therapy to prevent the emergence of isoniazid-resistant organisms. Sincerely yours,

POSTGRADUATE MEDICAL SCHOOL OF LONDON
LONDON, W. 12
ENGLAND

D. A. MITCHISON

December 15, 1953.

## VIRULENCE OF TUBERCLE BACILLI RECOVERED FROM PATIENTS TREATED WITH ISONIAZID

*To the Editor of The American Review of Tuberculosis:*

In the issue of THE AMERICAN REVIEW OF TUBERCULOSIS for October, 1953, Steenken and Wolinsky[1] reported investigations into the virulence of strains of *Mycobacterium tuberculosis* recovered from patients treated with isoniazid. They found that an artificially induced variant of H37Rv resistant *in vitro* to 50 $\gamma$ of isoniazid per ml. on egg medium was avirulent in guinea pigs. Of 8 strains isolated from patients who had not received isoniazid therapy, none was avirulent and only one showed a low degree of virulence, the remaining 7 having "good" or "fair" virulence by the authors' classification. Of 33 strains isolated from 25 patients during isoniazid therapy, the 5 which showed slight resistance to isoniazid *in vitro*, i.e., giving growth on 0.2 $\gamma$ per ml. of solid medium and only slight growth on 1.0 $\gamma$ per ml., were all classed as of "good" or "fair" virulence; of the 19 strains showing moderate resistance, i.e., giving growth on 1.0 $\gamma$ per ml., 7 showed "good" or "fair" virulence, while 3 were "slightly" virulent and 9 avirulent; of the 9 highly resistant strains tested, i.e., giving growth on 10.0 $\gamma$ per ml., 2 showed "good" virulence, 2 were only "slightly" virulent, and 5 were avirulent. The authors therefore conclude that "the phenomenon of loss of virulence of tubercle bacilli for guinea pigs is related to other factors in addition to the development of isoniazid resistance." But it may still be true that all pure cultures of tubercle bacilli highly resistant to isoniazid are avirulent for guinea pigs for the following reasons.

The organisms used in the experiments described by Steenken and Wolinsky were either from primary cultures of sputa or from the original sputum concentrates. In either case, therefore, they were not from a pure culture and may have contained organisms of

[1] STEENKEN, W., JR., AND WOLINSKY, E.: Am. Rev. Tubere., 1953, *68*, 548.

TABLE 1

VARYING DEGREES OF ISONIAZID RESISTANCE IN TUBERCLE BACILLI ISOLATED FROM SPUTUM FROM PATIENTS DURING ISONIAZID THERAPY

| PATIENT | DAYS OF ISONIAZID THERAPY | | PER CENT OF TOTAL ORGANISMS RESISTANT TO ISONIAZID (γ PER ML.) | | | | | | ROUTINE RESISTANCE TEST* |
|---|---|---|---|---|---|---|---|---|---|
| | First course | Second course | 0.05 | 0.25 | 1.0 | 4.0 | 16.0 | 64.0 | |
| | | | | | | | | | γ *per ml.* |
| J. L. | 168 | 160 | 100 | 60 | 8.0 | 0.1 | None | None | 10.0 |
| J. G. | 105 | 200 | 100 | 17 | 0.05 | 0.001 | None | None | 1.0 |
| M. M. | 101 | 89 | 100 | 100 | 75.0 | 10.0 | 2.5 | 1.0 | 50.0 |

* Method as used in the Medical Research Council (Great Britain) Isoniazid Trial, Lancet, 1953, *2*, 213.

varying degrees of resistance. We have been investigating the proportion of organisms of varying degrees of isoniazid resistance in any given specimen of sputum from patients showing isoniazid resistance on the routine sensitivity test. Table 1 shows some of the results of such investigations. It will be seen from these figures that in none of the three patients showing "moderate" or "high" resistance by the classification of Steenken and Wolinsky were all of the organisms resistant to the concentration shown on the routine sensitivity test, i.e., although highly resistant organisms were present, there were also present considerable numbers of organisms of lower degrees of resistance even after long periods of isoniazid therapy. These results are on only a few cases, and all patients were on a second course of treatment. It may well be that in some cases a higher proportion of resistant organisms occurs. Nevertheless, this does not always seem to be true. It would therefore seem that a primary culture from a sputum, or a sputum concentrate, may contain a mixture of resistant and sensitive organisms.

That organisms resistant to isoniazid may vary in virulence proportional to their degree of resistance has been shown by Barnett and associates.[2] They used resistant strains which had been subcultured on medium containing isoniazid and which therefore did not contain any sensitive organisms. Their strain resistant to 200 γ of isoniazid per ml. was shown to be avirulent for guinea pigs and of low virulence for mice. Strains of lower degrees of resistance possessed intermediate or full virulence. The results of any virulence tests on mixed cultures might, then, depend on the proportion of resistant organisms present and the degree of resistance. Such heterogeneous cultures may account for the variations in virulence found by Steenken and Wolinsky[1] in cultures giving "high" resistance *in vitro*.

Previous works referred to by Steenken and Wolinsky[1] as evidence that not all tubercle bacilli highly resistant to isoniazid *in vitro* are avirulent are open to some comment. Mitchison[3] has found that at least some isoniazid-resistant strains which are avirulent for guinea pigs are virulent for mice. Therefore, the work of Karlson and Ikemi[4] on mice and Goulding and associates[5] on rabbits does not seem applicable to the present discussion.

[1] BARNETT, M., BUSHBY, S. R. M., AND MITCHISON, D. A.: Brit. J. Exper. Path., 1953, *34*, 568.

[3] MITCHISON, D. A.: Adaptation in Micro-organisms, Cambridge University Press, England, 1953.

[4] KARLSON, A. G., AND IKEMI, Y.: Proc. Staff Meet., Mayo Clin., 1952, *27*, 373.

[5] GOULDING, R., KING, M. B., KNOX, R., AND ROBSON, J. M.: Lancet, 1952, *2*, 69.

TABLE 2

ISONIAZID RESISTANCE OF TUBERCLE BACILLI ISOLATED FROM VARIOUS SITES IN A LUNG FROM A PATIENT WHO HAD RECEIVED 46 DAYS OF ISONIAZID ALONE OR IN COMBINATION WITH DRUGS TO WHICH HER ORGANISMS WERE SUBSEQUENTLY FOUND TO BE RESISTANT.

| SOURCE OF CULTURE | ISONIAZID RESISTANCE* |
|---|---|
| | γ per ml. |
| Sputum 10 days preoperative | Sensitive |
| Wall of cavity in left upper lobe | 0.2 |
| Contents of cavity in left upper lobe | 10.0 |
| Small caseous focus in left upper lobe | 0.2 |
| Swab from left upper lobe bronchus | 50.0 |
| Swab from lingular bronchus | 0.2 |
| Cavity contents from apical segment of left lower lobe | 5.0 |

* Method as used in the Medical Research Council (Great Britain) Isoniazid Trial: Lancet, 1953, *2*, 213. (*By courtesy of Drs. F. W. A. Turnbull and A. T. Wallace*)

Barnett and associates[6] reported virulence in resistant strains in guinea pigs. However, the two resistant strains from patients which they used were from the primary cultures from sputa and were resistant to only 1.0 γ of isoniazid per ml., i.e., they were neither pure cultures nor highly resistant. Although resistances on solid medium were not given for the two artificially induced resistant strains, they would seem to have been of only "moderate" resistance and heterogeneous since the authors refer to a culture isolated from one of the infected guinea pigs as being "slightly more resistant (than the original strain), yielding a confluent growth on 1.0 γ per ml. and innumerable colonies on 5.0 γ per ml." From the later results[3] it is likely that these organisms would show some degree of virulence.

A recent paper by Peizer[7] reports virulence of highly isoniazid-resistant tubercle bacilli isolated from patients. But again primary cultures were used, and no sensitivities were carried out on any of the guinea pig lesions. The dissemination may, therefore, have been due to sensitive or partially resistant organisms.

Finally, in their discussion, Steenken and Wolinsky[1] refer to the observation that "patients whose bacilli demonstrate very slight or no virulence for guinea pigs may nevertheless undergo serious exacerbations of disease." Since these organisms may be virulent for laboratory animals other than guinea pigs, it seems reasonable that they may also be virulent for man. Alternatively, the organisms throughout the lung may not be of the same degree of resistance as those excreted in the sputum. Work done in this unit[8] on the isoniazid resistance of tubercle bacilli isolated from various lesions in lungs removed from patients who had received isoniazid therapy suggests that the degree of resistance may vary from one lesion to another. The results of one such case are shown in table 2. It will be seen that, although tubercle bacilli from the sputum ten days preoperatively appeared sensitive to isoniazid by the routine sensitivity test, cultures from various lesions showed resistances varying from 0.2 γ per ml. to 50.0 γ per ml., a range including

[6] BARNETT, M., BUSHBY, S. R. M., AND MITCHISON, D. A.: Lancet, 1953, *1*, 314.
[7] PEIZER, L. R., WIDELOCK, D., AND KLEIN, S.: Am. Rev. Tuberc., 1953, *68*, 290.
[8] TURNBULL, F. W. A., AND WALLACE, A. T.: Personal communication to the writer.

both the fully virulent and the avirulent strains reported by Mitchison[3]. These results are similar to those found by Canetti and Saënz[2] for streptomycin-resistant organisms. It may be, therefore, that the exacerbations referred to by Steenken and Wolinsky[1] are due to less resistant but more virulent organisms from a closed lesion which is not contributing tubercle bacilli to the sputum.

The problems of the virulence of highly isoniazid-resistant tubercle bacilli have yet to be elucidated, but it would seem desirable that more work should be done on pure cultures.

Department of Tuberculosis and Diseases of the Respiratory System
University of Edinburgh
Southfield Hospital
Liberton, Edinburgh

Sheila M. Stewart

December 11, 1953.

[2] Canetti, G., and Saënz, A.: Ann. Inst. Pasteur, 1951, *80*, 238.

# *Books*

ANNUAL REVIEW OF MEDICINE, Volume 4. Pp. 452, Annual Reviews, Inc., Stanford, California, 1953, Price, $6.00.

This comprehensive volume is a valuable extension of this series of reviews covering the recent literature in various phases of medicine. As is the stated intention of the Editors, the articles in general do not attempt to cover the entire scope of the subject undertaken, but rather the majority of them emphasize certain pertinent aspects of each topic. Far from being merely recitals of the current work in the field, each review is an integrated treatise on the particular subject or subdivision thereof; and, although the developments of the past two or three years are especially stressed, the material is drawn when necessary from earlier work, so that in most instances each review forms an interesting, fairly complete essay. The literature covered in these articles extends through the early fall of 1952, and each review provides an extensive bibliography.

The extent of this volume can best be illustrated by listing the subjects reviewed and their authors. The topics include Infectious Disease by W. S. Tillett; Diseases of the Gastrointestinal Tract by G. Cheney and C. P. Callaway; Diseases of the Cardiovascular System by R. J. Bing; Diseases of the Kidneys by F. Hinman, Jr.; Diseases of the Reticuloendothelial System and Hematology by L. Whitby; Nutrition by D. P. Cuthbertson; Allergy by J. Ferrebee; Neoplastic Diseases: Some Metabolic Aspects by G. B. Mider; Diseases of the Female Reproductive System by C. F. Fluhmann; Vascular Diseases of the Brain by R. Adams and H. M. Vander Eecken; Psychiatry by T. A. C. Rennie; Diseases of the Respiratory System by L. F. Davenport; Physical Agents and Trauma: Burns and Freezing by H. N. Harkins; Radiology by H. M. Wittenberg; Diseases of the Bones and Joints by J. H. Bauer; Obstetrics by F. F. Snyder; Tropical Disease by E. W. Dennis and D. A. Berberian; Plastic Surgery: Wound Care and Skin Grafting by L. T. Byars and P. Randall.

This material, accordingly, presents items of interest to the physician and surgeon alike, and it includes valuable information in fields of more general interest, as nutrition and neoplastic disease. The reviewers represent authorities from numerous major institutions in the United States, geographically widespread, and from England and Scotland. In general, the references are from the literature in the English language, but an attempt is made in most instances to cover recent, important developments published in other languages.

Following these articles is an Annotated List of Reviews of Medicine, most of which were published between October, 1951, and November, 1952, compiled by E. M. MacKay. These cover an even broader scope than the organized reviews of this volume and, although additional reviews in most of the above fields are cited, the annotations range from Diseases of the Teeth to Public Health. Careful author and subject indices for all portions of the work are found at the end of the volume.

W. N. CHRISTENSON
New York, New York

ADJUSTMENT TO PHYSICAL HANDICAP AND ILLNESS: A SURVEY OF THE SOCIAL PSYCHOLOGY OF PHYSIQUE AND DISABILITY. By Roger G. Barker, in collaboration with Beatrice A. Wright, Lee Meyerson, and Mollie R. Gonick. Pp. 440, Social Science Research Council, 230 Park Avenue, New York 17, 1953.

This lengthy monograph is a revision of a volume first published in 1946 by the Social Science Research Council, whose purpose is to plan and promote research in social fields. The authors are primarily concerned with the "*somatopsychological* relation between physique and behavior. This relation is concerned with those variations in physique that affect the psychological situation of a person by influencing the effectiveness of his body as a tool for actions or by serving as a stimulus to himself or others."

Those research studies which pertain to the somatopsychological problem have been surveyed and evaluated and constitute the major portion of the presentation in each of the following areas: physical size, muscular strength and motor ability, orthopedic disabilities, tuberculosis, auditory impairments, visual impairments, and acute illness. A chapter is devoted to each of these physical variations. At the end of each chapter appears a research summary—a list of studies pertaining to the disability under discussion.

The chapter which deals with the psychology of the tuberculous patient is concerned with the several particular problems frequently mentioned throughout the literature, i.e., that patients with pulmonary tuberculosis are characteristically cheerful and optimistic, that the tuberculous patient shows an increase in sexual urge and increase in intellectual acuity, the idea that the behavior of tuberculous persons is due to intoxication from the products of the metabolism of the tubercle bacillus versus the idea that behavior results from the peculiar psychological situation in which this disease places the patient.

A review of the studies (a meager number of which approach acceptable standards of scientific inquiry and reporting) indicate the following:

1. A great variety of behavior is exhibited by tuberculous persons; there is no unique behavior of the tuberculous patient.
2. There are indications that some kinds of behavior are more frequent than in healthy populations, although exactly comparable data are not available.
3. Anxiety, mild neuroticism, neurasthenia, and discontent are frequently found.
4. Euphoria is infrequently reported and, when it is, investigators question whether it is genuine or in the nature of a protective mechanism.
5. Data on sex drive are inadequate, but do not support the theory of increased sex drive.
6. There is no evidence that tuberculosis has a regressive effect upon intelligence; the bulk of the data supports the tentative hypothesis that tuberculosis may be associated with a slightly increased level of intellectual functioning.

There was found to be no direct experimental evidence on the question of what are the effects on behavior of being in a psycho-physical and psycho-social situation of having tuberculosis. However, this review refers to many studies around the questions of patients' reactions to diagnosis and treatment, both in hospitals and sanatoriums, which relate to the original question. The authors conclude that a great diversity of behavior is expected in tuberculous persons because of differences in the relevant variable in specific cases. Nevertheless, they add: "some constellations of concrete factors would appear to be very common, giving rise to such frequently recurring behavior as: conflict, instability, emotionality, selfishness, limited interest, hypochondriasis, and preoccupation with sex even though the libido may be low. Basic anxiety overlaid with superficial serenity, in-

creased autistic behavior, suggestibility, and ambivalence toward family and medical staff."

Some speculations are made with regard to the best organization of sanatorium procedures along optimal social-psychological lines. However, it is pointed out that this area is one of the most neglected in social-psychological research.

The literature pertaining to the social, occupational, and physical conditions of ex-patients was examined, and there is a description of numerous studies which describe the ex-patient's situation as he sees it and as it has affected him. The authors generalize that the psychological situation of the ex-patient is similar to that of the adolescent in that he "occupies a marginal, overlapping position between an underprivileged minority group (tuberculous patients) and a privileged majority group (healthy individuals).

The importance of sheltered work experience (such as sheltered work shops) is pointed out. By this means, some of the feeling of "newness" of the occupational situation can be removed. The chapter concludes with a listing of types of sheltered employment available to ex-patients at the present time.

This volume would seem to be of value as a resource or reference book to those professional persons working with any of the specialized groups described. It is also of interest to those persons who would know more about this new approach to the relation between physique and behavior.

MARJORIE A. JONAS
New York, New York

RESPIRATORY DISEASES AND ALLERGY. By Josef S. Smul. Pp. 80, Medical Library Company, New York, 1953, Price, $2.75.

The author has attempted to cover the extensive field indicated in the title in a 72-page monograph. That this task has been performed to the author's satisfaction, if not to the reviewer's, and with a bibliography consisting of only one reference on a minor point, is explained in the preface: ". . . owing to the exclusion of many useless theories this small volume covers all diseases of the respiratory system." Accordingly, the monograph includes many of the author's own ideas and excludes much of what has been learned from the clinic and laboratory about allergy and immunology.

The monograph has been divided into three sections which cover allergic diseases, infectious diseases, and neoplastic diseases, respectively.

In the section on allergic diseases of the respiratory system the author lists twenty-three ailments, including such diverse conditions as "neurosis of the respiratory tract," acute recurrent suppurative and chronic atrophic rhinitis, sinusitis, and bronchitis, and bronchiectasis. He believes that all of these are of allergic etiology and attempts to reclassify them within this concept. For example, acute suppurative sinusitis, which has generally been considered infectious, the author reclassifies as "acute suppurative respirallergy" and states that "[it] may be *complicated* infection causing . . . suppuration in the sinuses" [italics ours]. While most allergists recognize the existence of allergy to bacteria or their products in certain patients, few, if any, would go so far as to consider all bacterial infections as allergic reactions.

On the subject of treatment of allergic rhinitis the author has come to the surprising conclusion that three substances, namely, ragweed, dust, and catarrhalis vaccine are "universal respiratory desensitizers," and treats all patients with these three materials regardless of the patient's clinical sensitivities. Needless to say, this approach is completely contrary to what we know of the specificity of antigens. The author states: ". . . . verifica-

tion or denial [of his results] can only be advanced by noting the results obtained and repeating the work described and not by inventing a theory explaining why such results are impossible." Since the author includes no protocols, no details or tabulations on his series of patients, and no figures which would indicate what his actual results were, repetition of his work would be difficult. However, such repetition is not necessary, since it is a common experience among allergists, including this reviewer, to find patients clinically sensitive to various combinations of allergens, including such a combination as ragweed and dust, who are treated with injections of the offending allergens with alleviation of these sensitivities, only to develop clinical sensitivities to other allergens while still under treatment. Indeed, allergists are aware that a poor response to treatment or a worsening of symptoms after apparent improvement is very likely to be due to the development of new sensitivities in the allergic patient.

Section II, devoted to infectious diseases of the respiratory system, includes a total of eight diseases, covered in brief. The paragraphs on pathology of each of these are particularly inadequate. Indeed, an indication of the inadequacy in this area may be found in the inclusion of infarction of the lung as one of the infectious diseases in this section.

Section III consists of two pages on neoplastic diseases of the lung.

MURRAY DWORETZKY
New York, New York

## BOOKS RECEIVED

EFFECT OF ACTH AND CORTISONE UPON INFECTION AND RESISTANCE. Edited by Gregory Schwartzman. Symposium held at the New York Academy of Medicine, March 27 and 28, 1952. Pp. 204, with illustrations, Columbia University Press, New York, 1953, Price, $5.50.

ATTITUDES TOWARD GIVING. By F. Emerson Andrews. Pp. 145, Russell Sage Foundation, New York, 1953, Price, $2.00.

TECHNIQUES OF TUBERCULIN TESTING AND BCG VACCINATION. Public Health Service Publication No. 182. Pp. 44, U. S. Government Printing Office, Washington, D. C., 1953, Price, 55 cents.

RECORDS AND REPORTS OF LOCAL HEALTH DEPARTMENTS. Public Health Monograph No. 15. By Olive G. Johnson. Pp. 89, U. S. Government Printing Office, Washington, D. C., 1953, Price, 45 cents.

SCIENTIFIC COMMUNICATIONS RESEARCH DEPARTMENT. Volume III. July 1952–July 1953. Brocades Research Department, Brocades–Stheeman & Pharmacia, Amsterdam, Netherlands, 1953.

STRAHLENSCHUTZ UND SONSTIGER ARBEITSSCHUTZ BEI DER MEDIZINISCHEN ANWENDUNG VON RÖENTGENSTRAHLEN. By Wilhelm Ernst. Pp. 97, with illustrations, Georg Thieme Verlag, Stuttgart, 1953.

FUNKTIONELLE ATMUNGSTHERAPIE. By Julius Parow. Pp. 126, with illustrations, Georg Thieme Verlag, Stuttgart, 1953.

*American Trudeau Society*

**Medical Section of the National Tuberculosis Association**

# *Obituaries*

## FLORENCE RENA SABIN

**1871 1953**

Dr. Florence Rena Sabin, a distinguished histologist, who contributed notably to the research program of the National Tuberculosis Association and was a member of its Committee on Medical Research from 1929 to 1948, died in Denver, Colorado, October 3, 1953.

Born in Central City, Colorado, on November 9, 1871, Dr. Sabin received her medical degree from Johns Hopkins University School of Medicine, Baltimore, in 1900. She was the first woman to be graduated in medicine from Johns Hopkins, as she was the first woman to teach there. She joined the staff as assistant in anatomy and later became associate, associate professor, and finally professor of histology.

In 1925, Dr. Sabin joined the staff of the Rockefeller Institute for Medical Research, New York, the first woman to become a staff member. At both Johns Hopkins and the

**Florence Rena Sabin**

Rockefeller Institute, Dr. Sabin conducted important investigations on the blood and blood-forming organs. One part of her work was a study of cellular reactions in tuberculosis. In recognition of this work she was awarded the Trudeau Medal of the National Tuberculosis Association in 1945.

After a long career as teacher and researcher, Dr. Sabin embarked upon a new field of activity. Returning to her native Colorado on her retirement from the Rockefeller Institute, she was named chairman of the health section of the state's post-war planning committee and manager of health for the city of Denver. Her accomplishments in the field of public health won her a Lasker Foundation award in 1951.

Dr. Sabin was a member of a number of scientific groups, including the National Academy of Sciences, the American Association of Anatomists, the American Association of Physiologists, and the Society of Experimental Biology and Medicine. She was one of the few honorary members of the National Tuberculosis Association and was also an honorary member of the American Association for the Advancement of Science and was a member emeritus of the Rockefeller Institute.

E.R.L.

## JOHN D. ADCOCK

### 1909 1953

It is with deep sadness that we note the death last May of Dr. John D. Adcock of the faculty of the University of Michigan and member of the Editorial Board of the REVIEW.

Despite the dragging hindrance of chronic ill-health throughout most of his professional life, Dr. Adcock had attained a notable position as a scientific investigator, as a teacher, and as a person.

Dr. Adcock was born on January 11, 1909, in Warrensburg, Missouri; he was the son of Mrs. Elizabeth Hayes Hartman and the late Dr. Delbert Claude Adcock. Dr. John Adcock received an A.B. degree from the University of Missouri in 1931, and his M.D. in 1935 from the University of Pennsylvania. His postgraduate training was divided between the University of Pennsylvania Hospital and the University Hospital at Ann Arbor, Michigan. This training was repeatedly interrupted by bouts of ill-health, and he spent considerable time as a patient and as a staff member at the Trudeau and the Gabriels sanatoriums at Saranac.

Following his postgraduate education, he was appointed as instructor in medicine at the University of Pennsylvania Hospital, where he remained for only one year, when he was offered and accepted an assistant professorship of internal medicine at the University of Michigan.

At the University of Michigan, Dr. Adcock built up a unit for research and teaching in the field of infectious diseases and antimicrobial therapy. Like so many other distinguished physicians who have been victims of tuberculosis, Dr. Adcock had a considerable—although by no means exclusive—preoccupation with this infection. His first investigations were with nontuberculous infections and had to do with sulfonamide therapy. He was already a well-recognized expert in the field of sulfanilamide and penicillin therapy in January, 1945, when he started one of the initial investigations of streptomycin. His initial studies on the absorption and distribution of streptomycin in man represented an important contribution, and his papers on the subject are widely cited today. During these first months of 1945, Dr. Adcock was well aware of the potential of streptomycin as a chemotherapeutic agent in tuberculosis and actually started clinical investigation of streptomycin in tuberculosis only a short time after the initiation of the classical study (in humans) of Hinshaw and Feldman.

It was Dr. Adcock's sad fortune to have to abandon this study after only a few patients had been started on treatment because of a temporary scarcity of the drug and another episode of incapacitating illness. By the time he had recovered sufficiently to become again active as an investigator, more than two years had elapsed and streptomycin had become a generally accepted form of therapy. Dr. Adcock plunged into the field of chemotherapy in tuberculosis once again and made several noteworthy contributions. Among these were studies on the mechanics of the emergence of streptomycin-resistant strains of tubercle bacilli and one of the initial demonstrations of the antituberculous effectiveness of viomycin.

In these studies, as, indeed, in virtually all of the investigations he conducted, he would have to be content for prolonged periods of time to direct the experiments from his sickbed, with marked limitations on the number of trips he could make to the laboratory or clinic. It is inspiring to reflect that, despite these severe handicaps, Dr. Adcock was able to accomplish so very much.

In addition to his activities as a scientific investigator, Dr. Adcock served actively as a teacher of internal medicine and was held in high regard by his students. He repeatedly put his frail health to a strain by making the necessary trips to present papers at scientific meetings and to participate in the deliberations of scientific committees, notably those of the American Trudeau Society. In addition, during the last years of his life, he worked actively at a member of the Editorial Board of the REVIEW. In this capacity, as in his many other roles, he functioned with great effectiveness. His critical, but always good-humored, reviews of submitted manuscripts were of great assistance in carrying on the work of the journal.

Dr. Adcock was a Diplomate of the American Board of Internal Medicine, a Fellow of the American College of Physicians, a member of the Federation for Clinical Research, and a very active member of the American Trudeau Society.

He was a most stimulating companion and a fine conversationalist on both professional and nonprofessional subjects. From his boyhood days, he got great pleasure from hunting and fishing trips and was a highly proficient amateur in these fields. He was an excellent raconteur and delighted in telling "tall tales" in the finest traditions of American frontier humor.

Dr. Adcock is survived by his wife, the former Margaret Lucy Cowie, and by their son, John David. They are joined in mourning his death by all of the many people who knew him as a wonderful friend as well as a distinguished physician.

W. McD.

## HERBERT L. MANTZ

### 1896 1953

Herbert L. Mantz crossed the Great Barrier on December 14, 1953, in Kansas City, Missouri, at the age of fifty-seven. Stunned by the suddenness of his going and saddened by the significance of the happening were his wife, two daughters, two sons, his mother, and three sisters. Shocked were his numerous friends in city, state, and nation. Grieved were the many organizations that depended upon him for advice and encouragement.

As evidence of his broad influence in high places are his presidency, 1948–1949, of the National Tuberculosis Association, his services as one of the thirteen medical consultants to the Veterans Administration, his membership on four different occasions in the delegation to the Central American Congress Against Tuberculosis, and his participation at various times in medical meetings in England, Italy, France, Norway, and Sweden.

**Herbert L. Mantz**

His last honor and service were as official representative of the National Tuberculosis Association at the Union Latino-Americano de Sociedades de Tisologia (ULAST), December 5–10, 1953, in Caracas, Venezuela, from which he returned to die within a few hours.

National, state, and local medical organizations benefited by his interest and devotion. Among them: the National Tuberculosis Association and the American Trudeau Society by his service as an officer and member of important committees; the American Association of Industrial Physicians and Surgeons by his interest in its problems; and the American College of Chest Physicians, the Medical Society of Jackson County, the Southwest Clinical Society, and others by his devotion to their development.

Kansas City profited by his being a member of the Board of Trustees of its Baptist Memorial Hospital, his membership on the staffs of St. Luke's Hospital, St. Mary's Hospital, and Research Hospital. He was superintendent from 1926 to 1927 of the Municipal Tuberculosis Hospital, Leeds Station, and director for nearly twenty-five years of the city's tuberculosis clinics. He was for many years a deacon of the First Baptist Church.

Dr. Mantz was born in 1896 at West Plains, Missouri. He received his high school education in that city, his bachelor of arts degree in 1918 from the University of Missouri, and his degree in medicine in 1920 from the Jefferson Medical College in Philadelphia. He was a veteran of World War I and was devoted to the cause of the handicapped veteran in all phases of treatment and rehabilitation.

He was a member of Kappa Sigma college fraternity and of the Phi Beta Pi medical

fraternity. His hobbies were photography, stamp collecting, and horticulture. He was dynamic in his work, wide in his interests, faithful to his friends, and devoted to his family. His love of peonies and his success in cultivating them mirrors the soul of a man who will be missed as long as missing is a part of life.

E. B.

## JOHN DWIGHT DAVIS

**1896–1952**

John Dwight Davis died on December 15, 1952, at the age of 56, of pulmonary emphysema and cor pulmonale. He was the Chief of the Pulmonary Disease Service of the Veterans Administration Hospital, Long Beach; Associate Clinical Professor of Medicine, University of California School of Medicine at Los Angeles; and Senior Consultant in Medicine at Harbor General Hospital at Torrance.

He was born in Marion, South Dakota, on December 13, 1896, and graduated from the University of Illinois College of Medicine in 1922. He was a Fellow in medicine at the Mayo Foundation, Rochester, Minnesota, from 1926 to 1929, and later was on the faculty at the College of Medical Evangelists at Loma Linda and Los Angeles. For a time he was Medical Director of Olive View Sanatorium. He served in World War II as Lt. Colonel and Colonel in the Medical Corps of the Army.

Dr. Davis belonged to the American Medical Association and was a Fellow of the American College of Physicians and Diplomate of the Board of Internal Medicine. He was a Fellow of the American College of Chest Physicians and member of the American Trudeau Society.

C. B.

## CHRISTIAN WILLIAM NISSLER

**1889–1953**

Of Lansdowne, Pennsylvania; Jefferson Medical College of Philadelphia, 1919; Member of American College of Chest Physicians and American Medical Association; for many years member of Jefferson Medical College Faculty; formerly Medical Director of White Haven Sanatorium (Pennsylvania); died May 31, 1953, aged 64.

## PAUL EDWIN KOLB

**1884–1953**

Of Patchogue, New York; University of Maryland School of Medicine, Baltimore, 1912; Certified by American Board of Internal Medicine; Secretary–Past President of Suffolk County Medical Society; Past President of Associated Physicians of Long Island and Southside Clinical Society; Member of American College of Chest Physicians and American Medical Association; Major M.C., U.S.A., 1942; Veteran of World Wars I and II; Consultant to many local hospitals; for 33 years until 1949, Superintendent of Suffolk County Sanatorium, Holtsville, New York. Died June 14, 1953; aged 69.

## LEWIS ROBERT ROLL

**1910–1953**

Of Seattle, Washington; University of Chicago Medical School, 1941; Member of American Medical Association; Various hospital appointments until 1945–1947, when he was affiliated with Washington State Board of Health and Director of Oldercrest Sanatorium in Snohomish; Chief of Medical Service, Firland Sanatorium. Died June 20, 1953; aged 43.

### IRVING CHEIFETZ

**1904–1953**

Of New York City; Long Island College Medical School, 1930; various previous hospital appointments; Member of American Medical Association and Fellow of American College of Chest Physicians; affiliated with the Bronx and Morriania Hospitals and Seton Hospital. Died July 13, 1953, aged 49.

### JAMES FRANKLIN KARCHER

**1894–1953**

Of Mount Lebanon, Pennsylvania; University of Pittsburgh School of Medicine, 1925; Assistant Professor of Medicine, Hackett Medical College, 1927–1936; Sun Yat-Sen Medical College, Professor of Medicine, 1936–1942; Faculty University, Pittsburgh School of Medicine, 1942–1947; Staff Southside Hospital. Died July 7, 1953; aged 59.

### FRANK FERLAINO

**1899–1953**

Of New York City; Born February 1, 1899; Johns Hopkins University School of Medicine, 1927; American Board of Internal Medicine, 1945; Clinical Instructor of Public Health, New York Medical College, Flower and Fifth Avenue Hospitals; Veteran of World War I; Secretary of the Council on Postgraduate Medical Education, American College of Chest Physicians; District Counselor for the Industrial Medical Association; member of the American Medical Association, American Public Health Association; Medical Director of New York offices of General Motors; affiliated with Roosevelt Hospital; Medical Consultant to Stony Wold Sanatorium; Associate Editor of *Industrial Medicine and Surgery*. Died September 2, 1953; aged 54.

# NOTES

## 50TH ANNIVERSARY MEETING OF THE NATIONAL TUBERCULOSIS ASSOCIATION

The 50th Anniversary Meeting of the National Tuberculosis Association—first of the voluntary groups organized on a nationwide basis to fight a specific disease—and the 49th Annual Meeting of its medical section, the American Trudeau Society, will be held May 17–21, in Atlantic City. The Ambassador, Chelsea, and Ritz Carlton Hotels will be used.

Beginning Monday morning, May 17, there will be medical sessions on basic considerations in tuberculosis; nontuberculous pulmonary diseases; environmental considerations in tuberculosis; laboratory developments; surgery in the treatment of tuberculosis; and nonpulmonary tuberculosis, and panel discussions on the epidemiology of cancer of the lung, the treatment of tuberculosis in infants and children, and changing concepts and modern treatment of tuberculosis. There will also be a number of small seminars on various aspects of tuberculosis treatment and research.

The general theme of the anniversary meeting will be "The Challenge of the Future." There will be a number of general sessions, the first of which will be Monday afternoon, when the program will be built around "The People Against Tuberculosis—in Medicine, in Government, in Voluntary Agencies."

## SECOND WORLD CONGRESS OF CARDIOLOGY

The Second World Congress of Cardiology and the 27th Scientific Sessions of the American Heart Association will be held Sunday, September 12 through September 17, 1954, at the National Guard Armory, Washington, D. C.

For further information write to Dr. L. W. Gorham, Secretary-General, Second World Congress of Cardiology, 44 East 23 Street, New York 10, New York.

## CHEST SYMPOSIUM FOR GENERAL PRACTITIONERS IN SARANAC LAKE

The third annual Symposium on Tuberculosis and Other Chronic Pulmonary Diseases for General Practitioners will be held in Saranac Lake, New York, from July 12 through July 16, 1954. It is approved by the American Academy of General Practice for twenty-six hours of formal credit for its members.

The Symposium is sponsored by the American Trudeau Society, the Saranac Lake Medical Society, and the Adirondack Counties Chapter of the New York State Academy of General Practice. The registration fee is $40 for A.A.G.P. members and $50 for nonmembers.

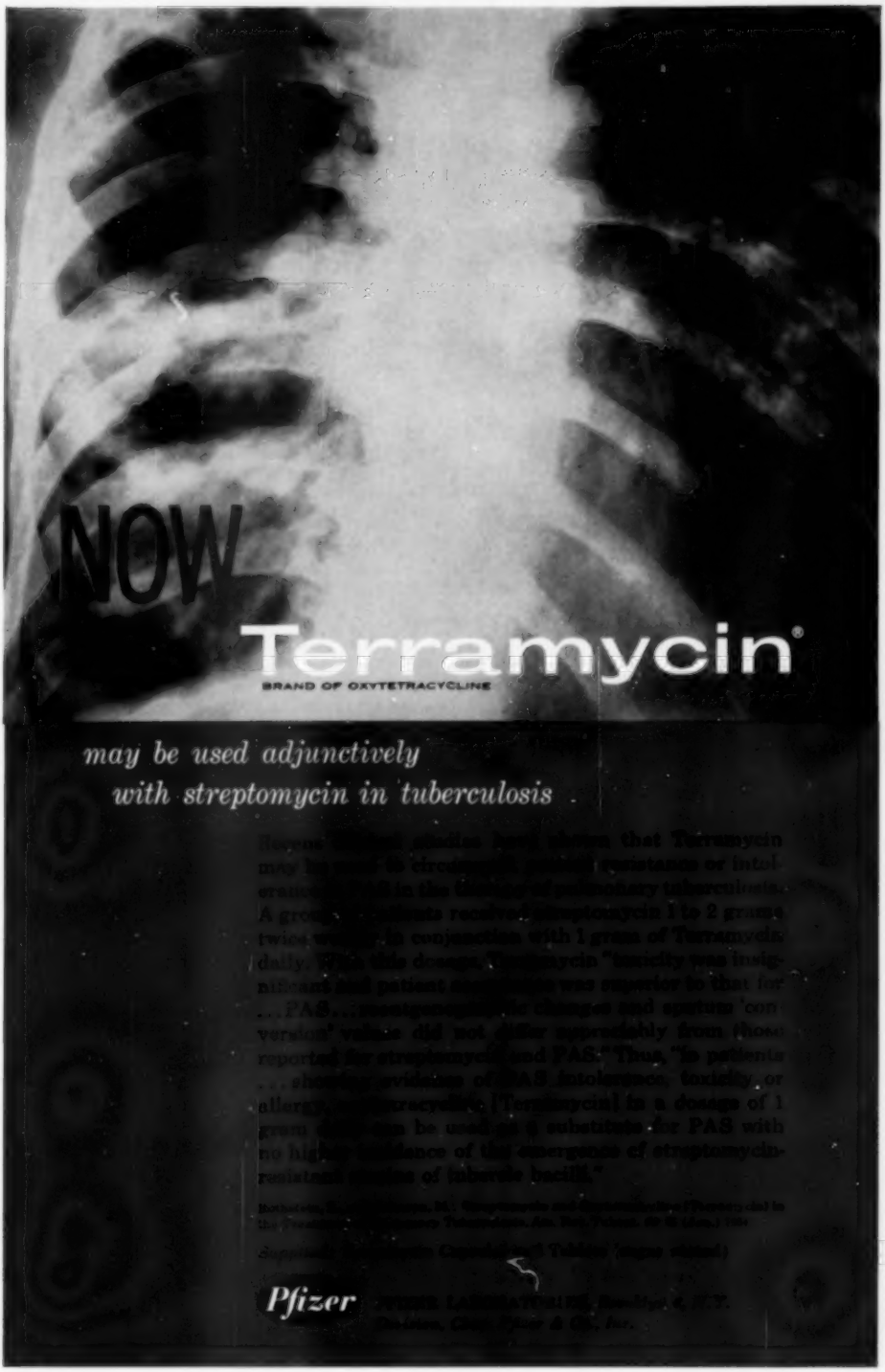
The scope of this year's Symposium has been broadened to cover chest diseases other than tuberculosis. Included in the course will be discussions of the diagnosis and treatment of nontuberculous pneumonias, pulmonary cancer, lung abscess, fungus diseases, bronchiectasis, sarcoid, cystic disease, emphysema, and the pneumoconioses.

Guest lecturers include Dr. D. Ewan Cameron, Professor of Psychiatry, McGill University, Montreal, and Dr. Donald Miller, Assistant Professor of Thoracic Surgery, University of Vermont Medical School, Burlington, Vermont.

Complete information concerning this program can be obtained by writing to Dr. Richard P. Bellaire, Chest Disease Symposium, P. O. Box 2, Saranac Lake, New York.

## APPOINTMENT OF DR. ARTHUR J. VORWALD

Dr. Arthur J. Vorwald, former director of the Saranac Laboratory of the Trudeau-Saranac Institute, Trudeau, New York, has been appointed first professor of occupational medicine and director of the Institute of Occupational Medicine at Wayne University, Detroit, Michigan.

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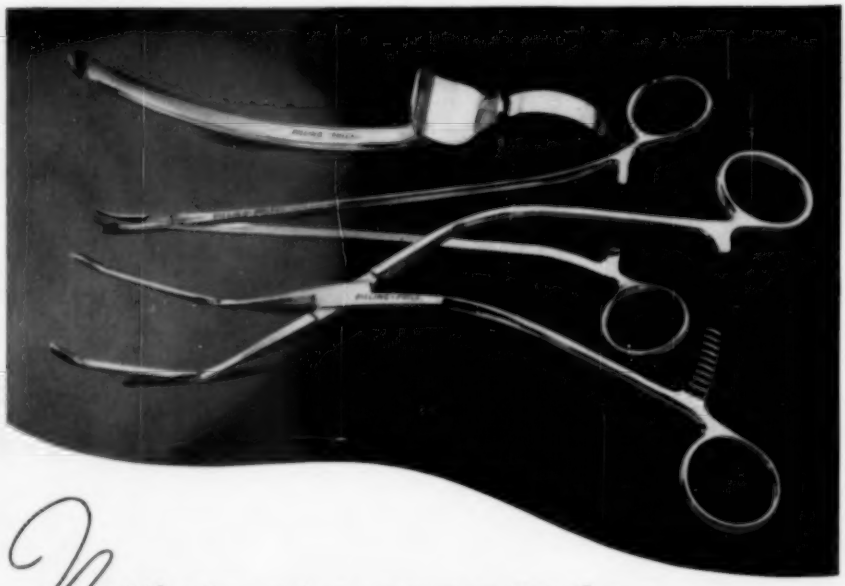

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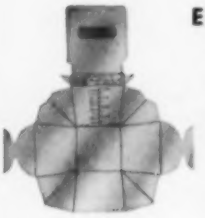

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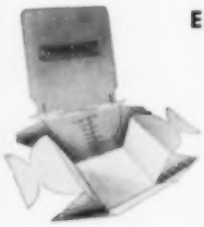

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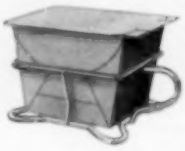

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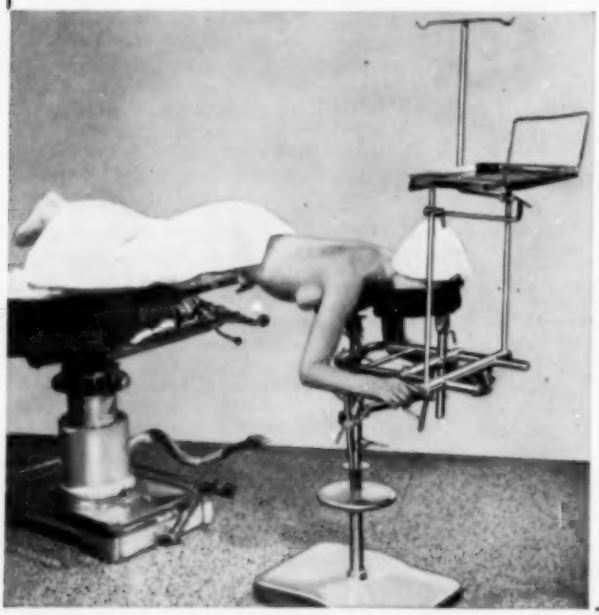

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## CONTENTS

*(Continued from cover 1)*

Notes:
An Appraisal of the Theories of Pleuritic Pain. HARRY A. BRAY ..... 634
Methods of Drainage Following Pulmonary Resection. JOHN D. STEELE AND BARNEY B. BECKER ..... 636
Tuberculosis Mortality Rates Among World War II Veterans (A Screened Population) for the Years 1948 Through 1952. LEO V. SCHNEIDER AND DAN I. ROSEN ..... 638
Correspondence: Virulence of Isoniazid-Resistant Tubercle Bacilli. D. A. MITCHISON; Virulence of Tubercle Bacilli Recovered from Patients Treated with Isoniazid. SHEILA M. STEWART ..... 640
Books ..... 645
American-Trudeau Society:
Obituaries ..... 649
Notes ..... 655
Abstracts ..... 81

## 50th ANNIVERSARY ★ NATIONAL TUBERCULOSIS ASSOCIATION
## Annual Meeting, Atlantic City, New Jersey, May 17-21, 1954

The 50th Anniversary of the National Tuberculosis Association will be celebrated at its Annual Meeting to be held in Atlantic City, New Jersey, May 17-21, 1954.

Three adjacent hotels, the Ambassador, Chelsea and Ritz-Carlton, will serve as headquarters. Sessions and conferences will be divided among the three hotels with no one hotel marked exclusively for medical or community service programs.

Applications for rooms should be made directly to the hotel of choice. In the event that the hotel is unable to provide the desired space, requests will be turned over to the Convention Bureau, which will clear requests with another one of the cooperating hotels.

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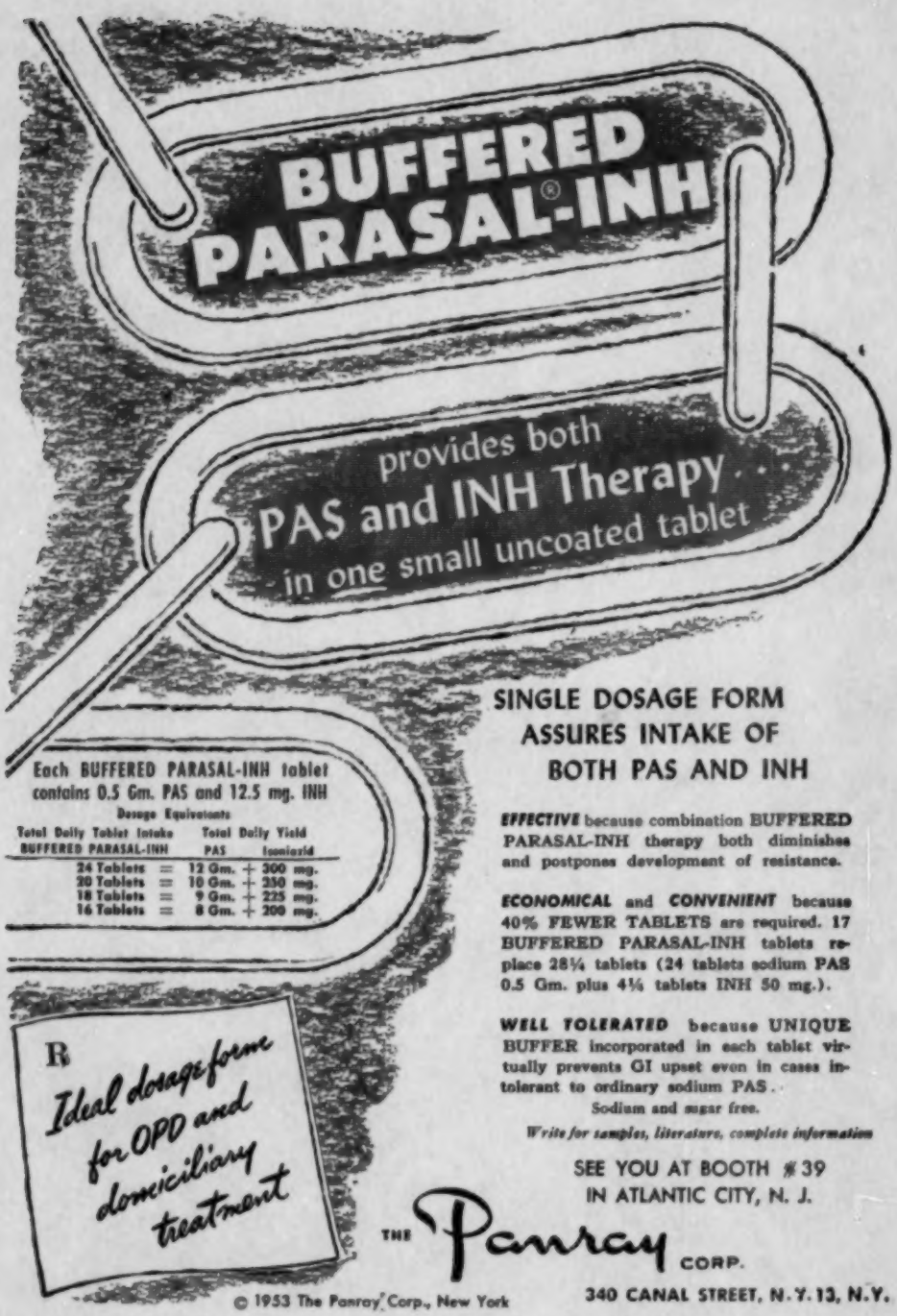


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